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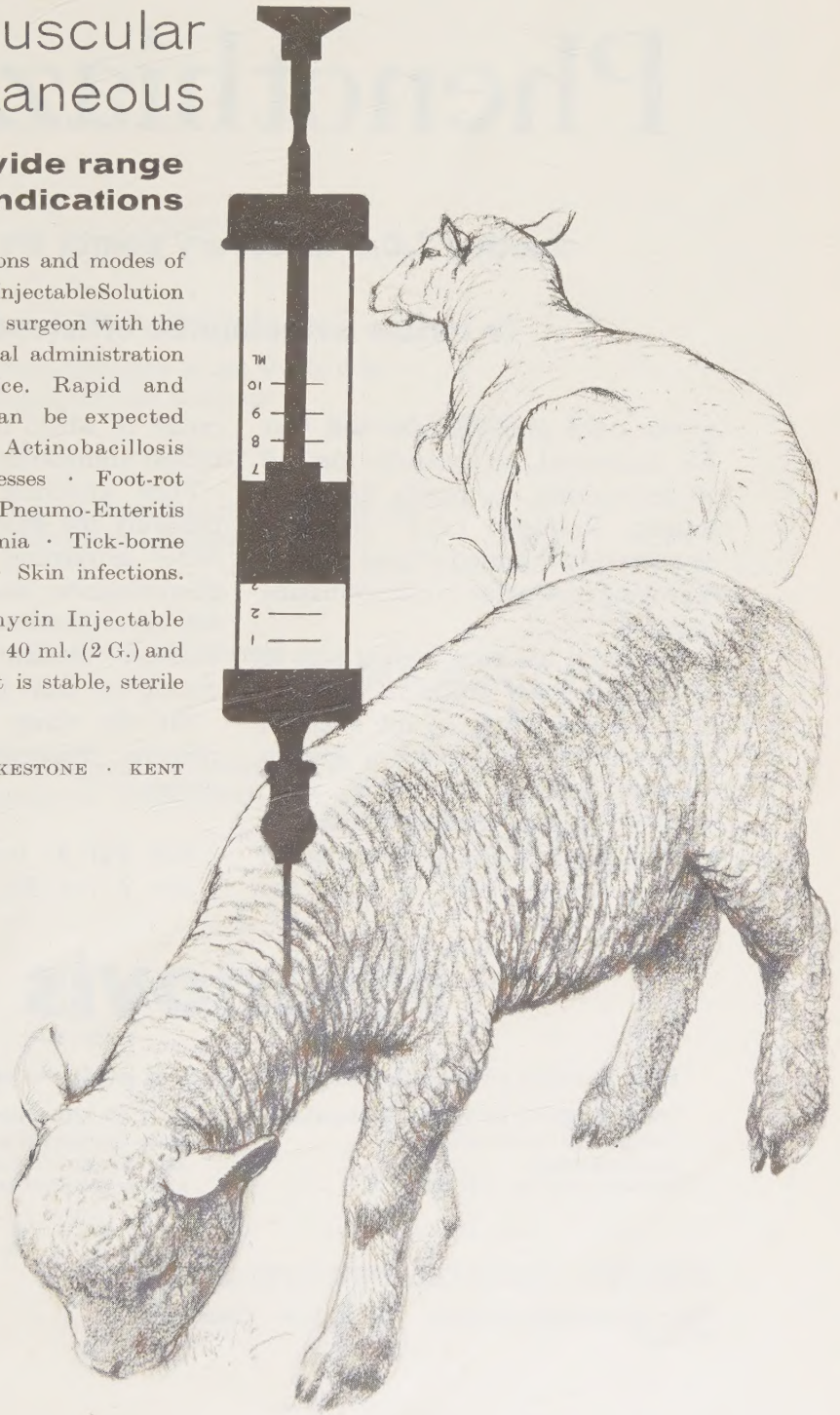

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COMMONWEALTH BUREAU OF ANIMAL HEALTH

VETERINARY REVIEWS AND ANNOTATIONS

Volume 5 October, 1959 *Part 2*

Recent Developments in the Control of Blowfly Strike in Sheep

BY

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INTRODUCTION

Blowfly strike has appeared sooner or later in every country which has established a sheep industry, and varies considerably in nature and incidence from one country to another. It appears to be most severe in Australia, South Africa and Great Britain. In Gt. Britain, not only are large numbers of sheep struck, but it continues over a long period from summer to late autumn and demands surveillance when other farm duties are pressing. In New Zealand and India the problem is a mild one, while little if any strike occurs in Europe outside Gt. Britain. In America, infestation with "fleeceworms" is overshadowed by much more disastrous screw worm infestations.

It is not surprising, therefore, to find that most of the research on blowfly strike has come from Australia, Gt. Britain and S. Africa where it is most severe. Research has led to a better understanding of the problem and the development of effective control measures. The work may be broadly divided into two phases. The first was concerned mainly with identification of the species of flies responsible, their distribution, and the conditions necessary for strikes to develop, both in relation to the climate and to the host itself. From this arose the various measures for reducing sheep susceptibility. The second phase, from about 1946 onward, might be termed the insecticide phase, and is the main subject of this review. Such a review appears timely, since the recent appearance of flies resistant to some insecticides will place certain limitations on their future use.

PERIOD PRIOR TO 1946

By 1946 the species of flies responsible for strike had been identified and classified into primary flies which initiated strikes and secondary or tertiary flies which subsequently invaded established lesions. It had also been shown that even when climatic conditions favoured the abundance and activity of flies, suitable conditions on the sheep were necessary for oviposition and the subsequent development of strikes. The most important factor was the presence of moisture.

It was shown that in crutch strike, "scald" (a dermatitis caused by irritation by urine and faeces) with its associated bacterial growth, tended to keep the skin moist thus providing ideal conditions for strikes to develop. With body strike, rain initiates the development of fleece rot which in turn predisposes sheep to attack by flies. With poll strike in rams, exudates at the base of the horns provide suitable conditions.

Preventive measures were therefore directed towards reduction of inherent predisposition of sheep, reduction of immediate susceptibility, and the reduction of fly populations.

In countries where the Merino is the dominant breed and scalding is associated with large skin wrinkles and folds in the crutch area, reduction of predisposition was sought either by selection and breeding of animals free from skin wrinkles or by the mechanical removal of crutch and tail skin wrinkles. The latter method, originally carried out by a grazier, Mr. Mules, and later developed by workers of the Council for Scientific and Industrial Research and state agricultural departments in Australia, has become known as the "Mules operation". Its efficiency, which reduces permanently the susceptibility of the sheep, has been recorded in various research papers and has generally proved more effective than selective breeding. In fact, it has been found that the strike rate in "mulesed" sheep is even lower than that in naturally plain-bodied animals.

Other measures aimed at reducing immediate susceptibility include crutching (shearing the wool from the breech area and hind legs) and the application of chemicals. Chemical control, the only means of combating body strike, was limited to the use of arsenical preparations such as calcium arsenite or sodium arsenite.

In attempts to reduce fly populations, both trapping and the destruction of carcasses proved to be quite ineffective.

While the selective breeding of plain-bodied sheep, "mulesing", jetting (power spraying so that the fleece and the skin become thoroughly saturated) and crutching were advocated in Australia and S. Africa, in Gt. Britain control was mostly by crutching and dipping in arsenical preparations. The latter gave only three or four weeks' protection, so repeated dippings were frequently necessary during the season of fly activity.

Before 1946 the only chemical used to protect sheep from strike was arsenic, while various dressings, mostly of limited efficacy, were used for treatment. Research on possible attractants and repellents had also met with little success.

RECENT WORK WITH ORGANIC INSECTICIDES

When the remarkable insecticidal activity of DDT and BHC became apparent, workers in various countries soon realized their potential value to the sheep industry and from 1945 their merits were intensively investigated. Since then, numerous other chemicals have been tested, of which dieldrin, aldrin and diazinon have become extensively used. To the conventional methods of application, dipping and jetting, have been added new devices which rely on the long lasting and diffusing properties of these chemicals in wool, and designed to make application easier and quicker.

Methods for Evaluating Test Chemicals. The difficulties associated with field tests for the critical evaluation of insecticides for blowfly control were first discussed by Macleod (1938) and later reviewed by Stones (1950). They include the uncertainty of fly activity during the test period, and the variation in individual susceptibility of sheep. The presence of insecticide on treated sheep also appears to affect to some extent the strike rate on controls running with them so that the true value of a chemical is masked.

Various laboratory and semi-field tests have been evolved to overcome these difficulties. Du Toit & Fiedler (1953) developed two types of *in vitro* test using first instar larvae. The first assessed the larvicidal properties of compounds after short duration contact. The larvae were placed on filter papers over a sintered glass filter and serial dilutions of chemicals applied for 5 sec., after which the filter papers were inverted on to liver slices in jars and the mortality of larvae recorded at 24, 48 and 72 hours. The second assessed the effect of long duration contact with larvicides. In this, the chemicals in wettable powder form were mixed with normal serum in concentrations from 1,000 to 0.004 p.p.m. The serum plus insecticide was then absorbed on pledgets of cotton wool on which the larvae were placed, and mortality recorded at intervals up to 72 hours.

In an extension of this method using segments of staples of wool from treated sheep instead of the cotton wool, they demonstrated that certain chemicals could diffuse down the fibre from the treated wool to the continually expanding zone of new growth and concluded that the duration of protection afforded by an insecticide is dependent on its larvicidal activity and its power of diffusion.

In vitro tests reported by Shanahan (1946) involved the immersion of prepupae and noting the mortality and subsequent emergence of flies after pupation. Du Toit & Fiedler (1952) also used third stage larvae to assess the larvicidal properties of a number of chemicals.

Two *in vivo* techniques have been developed to assess the activity of compounds when applied to sheep. The first determines the effect against the adult fly and involves the exposure of treated sheep to a dense population of ovigerous primary flies in an insectary. The sheep are made "temporarily susceptible" to flies by means of an attractant (Hobson, 1936). The incidence of oviposition is taken as a measure of the comparative efficiency of compounds. This method has been used by

Stones (1950) and Stones et al. (1954), Waterhouse & Scott (1950) and Riches & O'Sullivan (1955). The second technique, which assesses the larvicidal properties of a compound, was developed from the work of MacLeod (1937), who showed that when first stage larvae were placed on the abraded skin of sheep, then covered with a moist cotton wool plug, strikes developed with a high degree of certainty. Treated sheep are "challenged" with larvae in this way at regular intervals, and the time when strikes are obtained in a certain number of sheep in a group is taken as the period of protection afforded by the chemical. This method, which has become known as the larval implant technique, has been used extensively by various workers (Stones, 1950; Stones et al.. 1954; Riches & O'Sullivan, 1955, 1957; Wright et al., 1957; du Toit & Fiedler, 1953a, b; Fiedler & du Toit, 1956).

It must be emphasized that the results obtained from this type of test are essentially comparisons between compounds tested at the same time. The periods of protection thus estimated are not necessarily those which can be expected under field conditions when such factors as wool staining, scouring, intensity of fly activity and differing climatic conditions may affect the results. By quoting these semi-field results out of their proper context, extravagant claims have been made for the efficacy of some compounds which have not been substantiated in practice.

DDT and BHC. The first reports on the use of DDT for blowfly control came from Gt. Britain, where a number of workers (Cragg, 1945, 1946, 1947; Harbour & Watt, 1945; Hughes et al., 1946, 1947; Lyle-Stewart, 1946; Stamp et al., 1948) obtained valuable protection for up to seven weeks by dipping in emulsions containing 0.5% DDT. In the course of this work it was noted that failures generally occurred in the crutch area first, but the strikes tended to be limited in their extent. In S. Africa higher concentrations of DDT (2.5 to 6%) were tested by jetting and gave somewhat longer periods of protection up to three months (du Toit & Goosen, 1949). Following insectary tests by Waterhouse & Scott (1950), in which DDT at 1 and 2% concentration was found to give 6–8 weeks' protection and an extended period of partial protection, Johnstone & Scott (1951) saturated the backs of sheep in a shower dip with the same concentrations of DDT. Immediately after treatment a severe fly wave occurred and excellent protection was afforded. It was considered that concentrations above 1.0% were unwarranted. In these trials very heavy rain fell within a few hours of treatment, but had no apparent effect on the protection afforded for the ensuing 24 days. The fly attack then abated, so that it could not be determined if longer protection would have been obtained.

The larvicidal activity of BHC against several stages of Lucilia spp. was shown both in vitro in the laboratory and on sheep by Shanahan (1946), Stones (1950) and Waterhouse & Scott (1950). In field trials, Shanahan (1948) obtained three weeks' protection against body strike with 0.06% gamma BHC when flies were very active. Later Shanahan & Morley (1948a) showed that BHC was very effective in preventing poll strike in rams. When the same workers (1948b) used BHC at concentrations of 0.03 and 0.06% gamma isomer for the prevention of crutch strike by

jetting, they obtained no better results than with calcium arsenite. BHC had an advantage over arsenical preparations because it could be safely applied to active strikes. These findings were in general agreement with those of British workers who found that BHC dips were about equal to those containing arsenic.

South African workers recommended much higher concentrations of BHC (0.5%) as a result of semi-laboratory tests in which 30 weeks' protection was obtained.

At this stage few precise data were available from comparative trials on the relative merits of DDT and BHC. Shanahan (1951) reported slightly better results with 1.0% DDT than with 0.06% gamma BHC. This trial was made under very severe conditions of fly attack when over 50% of the control sheep had body strikes. In further trials (Shanahan, 1953), 4.3% of sheep treated with 1% DDT were struck, compared with 14.1% in those treated with 0.06% BHC and 35.5% in control sheep.

The position then, in 1950–52, prior to the introduction of dieldrin and aldrin, was that in Gt. Britain DDT "maggot fly dips" were preferred, the S. African recommendations were for high concentrations of BHC, while in Australia the official recommendations (Joint Blowfly Control Committee, 1950) were: "In anticipation of a wave of body strike, for prevention use DDT or BHC (DDT for preference). Should a wave of body strike develop use BHC generously". For crutch strike the same recommendations applied, together with "mulesing" where applicable and crutching at the right time.

Dieldrin and Aldrin. At the 1952 meeting of the British Veterinary Association, Downing et al. (1952) presented a paper in which the merits of several insecticides were tabulated in relation to their persistence as anti-adult and larvicidal agents. Among these, a dieldrin emulsion (0.3%) was rated as having extremely good larvicidal action. Du Toit & Fiedler (1952) also demonstrated the activity of dieldrin and aldrin in this respect in laboratory tests, but considered that BHC gave superior results at the same concentration. Stones et al. (1954) disagreed with their findings on BHC, and reported outstanding results with dieldrin in both field and laboratory trials. Several workers have since confirmed the efficiency of dieldrin and aldrin in larval implant experiments (Riches & O'Sullivan, 1955; Wright et al., 1957).

When dieldrin and aldrin were used in the field their superiority to BHC and DDT was soon evident, and the latter were rapidly replaced.

Using dieldrin dips at a concentration of 0.05%, Stones et al. (1954) obtained 12–16 weeks' protection against body strike and 8–10 weeks' against crutch strike. Fourteen weeks' protection was obtained against body strike by Graham (1954) when 0.1% dieldrin was jetted along the mid-line of the back. Only slightly inferior results were obtained with 0.05% during a period of severe fly activity. In these trials, Graham found BHC markedly inferior to either dieldrin or aldrin (which were equally efficient).

For the prevention of crutch strike, Moule et al. (1955) found that either dieldrin or aldrin at 0.1% gave good protection for four weeks but by the sixth week the strike rate was similar to that in control sheep.

They also found that dieldrin at 0.05% was slightly better than aldrin at the same strength. In later Queensland trials these findings were substantiated (Moule et al., 1957) and dieldrin at 0.05% concentration gave much better results than arsenic at 0.8% concentration. Graham (1957a) reported that when lambs were jetted at marking time with either dieldrin or aldrin, some degree of protection was afforded when flies became active 10–17 weeks after treatment. The period of complete protection could not be assessed. Further, the strikes which did occur were limited in extent and this was particularly noticeable with dieldrin.

Dieldrin had been shown by du Toit & Fiedler (1953a, 1954) to diffuse down the wool fibre from the treated wool into untreated wool; this fact together with its excellent larvicidal and persistent properties suggested that a light application to the surface of the fleece might give satisfactory fly control. This has led to the development of low volume spray races, which are discussed in a later section.

The remarkable persistence of dieldrin in fleece was further demonstrated by Lipson & Hope (1955), who found that wool taken from sheep dipped three months previously at concentrations as low as 0.005 to 0.01% was still toxic to clothes moth larvae. More recently Harrison & Johnson (1958) failed to remove all the aldrin from wool even by soxhlet extraction for several days. These workers also considered that, although the loss of aldrin from the fleece was rapid during the first month after application, it then became more gradual, so that sheep treated in the autumn in England were still protected from fly strike in the following May.

Since 1955, dieldrin and aldrin have been used extensively for fly control in Gt. Britain, Australia, New Zealand and S. Africa, and when properly applied at adequate concentrations have given excellent results. The recommended concentration for aldrin has usually been twice that for dieldrin because of the former's lower cost.

In Australia both insecticides were used to about an equal extent, with about equal efficiency under most conditions. However, when fly strike was very severe or occurred a long time after treatment, dieldrin appeared to give better results. Unfortunately the appearance of strains of flies resistant to these chemicals may well place limits on their future use.

Diazinon. Fiedler & du Toit (1956) tested a number of organo-phosphorus insecticides and found diazinon outstanding as a larvicide. When applied to sheep at 0.5% concentration it gave 51 weeks' protection compared with 37 weeks with dieldrin under similar conditions. Riches & O'Sullivan (1957) confirmed these findings, obtaining 23 weeks' protection with 0.008% diazinon in one trial, and in another, 16–18 weeks with 0.02%. At the same time aldrin at 0.025% concentration gave only 7–9 weeks' protection. Wright et al. (1957) obtained results which indicated that diazinon might afford protection over a whole blowfly season. All these trials, however, were done by the larval implant technique under semi-field conditions.

There is little evidence available that such long periods of protection are given under field conditions. The scarcity of controlled field

experiments with diazinon is due, no doubt, to the fact that when diazinon was first introduced into Australia, seasonal conditions were such that they were not conducive to severe fly activity. Moule et al. (1957) found that in Queensland diazinon and dieldrin gave comparable results against crutch strike. Skerman & Pryor (1957) suggested that diazinon at 0.02% was as effective as dieldrin at 0.04% concentration, but their trials were done in a period of low fly activity and under the milder climatic conditions of Victoria.

While dieldrin gives somewhat less protection in the field than in the laboratory, the discrepancy is not so marked as with diazinon. It has been suggested that the latter is lost at high temperatures because of its lower vapour pressure.

A peculiar effect, which was noted in larvicide tests by Fiedler & du Toit (1956) with organo-phosphorus compounds, was that the larvae which survived grew more rapidly than untreated larvae. The writer has also observed that when diazinon treatments on sheep fail, they do so abruptly, giving only a very brief period of partial protection and the resulting strikes thrive rapidly. This is in contrast to dieldrin with which the period of complete protection is followed by a period of partial protection, during which strikes are confined to a small area and the larvae grow poorly.

It is generally considered, however, that when used at concentrations from 0.02–0.04%, diazinon will give effective control of fly strike.

Other Insecticides. Although only DDT, BHC, dieldrin, aldrin and diazinon have become established for field use, numerous other insecticides have also been tested. To date they have all been rejected, either on account of their inferiority compared with those in common use, their toxicity for mammals or their deleterious effect on wool. Graham & Gaines (1948) showed that toxaphene was more effective than chlordane or BHC against fleece worm larvae. Waterhouse & Scott (1950) found chlordane and toxaphene active but considered them inferior to DDT when assessed against adult flies.

Du Toit & Fiedler (1953a) tested a wide range of compounds by bioassay methods and placed them in three groups -- those giving up to 10 weeks' protection (DDT, methoxychlor and TDE), those giving between 10 and 20 weeks' protection (chlordane or toxaphene) and those giving over 30 weeks' protection (dieldrin, aldrin and BHC). Later, these workers (1953b) compared dieldrin and aldrin with their stereo-isomers, endrin and isodrin. Isodrin gave excellent results, appearing to be slightly superior to dieldrin. However, both isodrin and endrin had deleterious effects on the wool and their use was not considered desirable. Moule et al. (1955) reported that endrin at 0.05% protected ewes against breech strike for 4–9 weeks and was about equal to dieldrin. Fiedler & du Toit (1956) also tested a number of organo-phosphorus insecticides, including malathion, Bayer 21/199, Bayer L13/59, EPN300, parathion and diazinon. Parathion and EPN300 gave good results but their high toxicity precludes their use; the results with diazinon were vastly superior to the other chemicals, so that, of these compounds, it became the insecticide of choice.

Riches & O'Sullivan (1957) found that malathion gave 10 weeks' protection compared with dieldrin (18 weeks) and aldrin (16–17 weeks) when used at the same concentration (0.25%). Limited trials in Queensland in 1957 on the control of crutch strike in weaner lambs indicated that Bayer L13/59 was of little value, while malathion at 0.05% was about equal to aldrin (0.1%) and diazinon (0.04%). Bayer L13/59 was also ineffective in larval implant tests (Anon., 1957). Larval implant experiments have indicated that D.D.V.P. (dimethyl dichloro vinyl phosphate) and the organic phosphorus compound "Nexion 1384" are also ineffective, while Bayer 21/199 at the relatively high concentration of 0.75% gave 16 weeks' protection (Anon., 1958).

The use of Dow ET–57 ("Korlan") has not been fully explored, but McGregor & Miller (1958) have reported that, in trials in Alabama, when used as a 0.5% spray, it gave complete freedom from fleece worms. At the same time 10% of the control sheep were affected. They also state that in further trials in Mississippi treatment of struck sheep with 0.5% "Korlan" gave a complete kill of larvae in one and a half hours and prevented re-infestation for 6–8 weeks. Lower concentrations also appeared promising.

Systemic Insecticides. The subcutaneous injection of sheep with aldrin, dieldrin or lindane in peanut oil did not give sufficiently promising results in preliminary trials to warrant further investigations on this form of administration, either for prevention or treatment of strikes (Anon., 1955). More recently, the activity of certain organo-phosphorus insecticides when used systemically in cattle for the prevention of warble fly damage, has renewed interest in this form of control. Marquardt & Hawkins (1958) used Dow ET–57 as a systemic for the treatment of fly-struck sheep with variable results. Larvae feeding on skin tissues were apparently affected but those in the wool were not. Little protection was afforded against recurrence. Systemic treatment, therefore, seems to hold little promise until such time as a highly effective larvicide, which is excreted in the skin and persists there, is available.

RESISTANCE

The first reports of resistance of L. cuprina to dieldrin were made by Shanahan (1958a). Dieldrin and aldrin had been in fairly general use in New South Wales for some three to four years, and while generally good results were still being obtained, in the spring of 1957 there were occasional reports of strikes occurring 7–14 days after sheep had been efficiently treated. Shanahan was able to show that two strains of adult flies were from 100–200 times as resistant to dieldrin as a susceptible strain and that their prepupae were at least ten times more resistant. Further work (Shanahan, 1958b) showed that this strain while being very heterogeneous, was also resistant to aldrin, but not to DDT, diazinon or BHC. More recent tests (Shanahan, private communication) have shown that it is resistant to BHC. Later field reports indicate that resistance is now prevalent throughout New South Wales, in southern Queensland, and to a lesser extent in the other States of Australia. O'Sullivan (private

communication) has tested strains of flies taken from active strikes on treated sheep in Queensland and found them resistant to dieldrin, aldrin, BHC, isodrin, endrin, chlordane and toxaphene. When investigated, none of the reports of possible resistance to diazinon has been confirmed, so that at the moment DDT and diazinon appear to be the insecticides of choice in those areas where dieldrin resistance has developed. Similar resistance has also been reported from S. Africa (du Toit, personal communication) but, to the author's knowledge, none has yet appeared in other countries.

BLOWFLY DRESSINGS

A great variety of remedies has been used for the treatment of struck sheep; arsenical compounds, copper sulphate, tars, oils, solvents such as petrol and kerosene, numerous "patent mixtures" and even earth. None could be said to meet the requirements of a satisfactory blowfly dressing. Some were fairly effective in removing maggots from the strike, but most were irritant, delayed healing and gave little or no protection against re-strike. Considerable progress resulted from S. African and Australian work in which dressings, based on boric acid, tar oils and bentonite (Lennox, 1941) or tar oils and cresol (Mönnig, 1943), proved to be highly effective and comparatively non-irritant. When suitable tar oils became unavailable, Waterhouse (1944) suggested the use of kerosene, orthodichlorbenzene and lysol to replace them, giving an effective dressing which became known in Australia as BKB.

However, these formulations were soon superseded when DDT and BHC became available. Since DDT proved to be a comparatively poor larvicide, orthodichlorbenzene was incorporated with it. Shanahan & Morley (1947) showed that BHC used as an emulsion at approximately 0.1% was an efficient dressing, while in S. Africa a 0.5% BHC wettable powder was recommended as being completely non-irritant (du Toit & Fiedler, 1952).

Since BHC dressings are bland, the practice has arisen in Australia of treating struck sheep without removing the affected wool. This makes treatment easy, saves time and also wool.

Dieldrin and aldrin preparations, although somewhat slower in action, were equally effective as dressings, but few special formulations marketed as jetting preparations were found to suffice.

More recently diazinon has been used in Australia with variable success. Some reports indicate that it may fail to prevent re-strike, and for this reason manufacturers have incorporated DDT with it. This failure is said to be due to loss of diazinon under high temperature conditions and perhaps also to the fact that when wool grease is leached out, as may occur in a strike, there is insufficient grease to hold diazinon in effective concentration.

Following the work of Lennox & Hall (1940), who showed that citronella oil was an effective repellent against L.cuprina, Johnstone (1951) tested it as a dressing for use on lamb marking wounds. He found that combined with boric acid it was superior to DDT, BHC and Huon pine oil, and later (1954) in an attempt to find a satisfactory substitute for citronella oil, Johnstone et al. showed that dibutyl phthalate gave good but

slightly inferior results to the natural product.

METHODS OF APPLICATION OF INSECTICIDES

Dipping has been widely used for the application of chemicals for fly control, particularly in Gt. Britain where the comparatively small size of flocks and the lack of emphasis on wool production has made it a practical proposition. It is wasteful of chemical and particular attention must be paid to maintaining the concentration of the insecticide, which tends to fall during dipping as a result of selective removal of both chlorinated hydrocarbon and phosphorus insecticides by the fleece. Jetting, on the other hand, in which fresh material is used continuously, minimizes wool staining and ensures that each sheep receives the required concentration of chemical on those areas which are most susceptible to fly attack. Many types of jetting plants are in general use but it seems that, irrespective of the equipment used, saturation of the fleece should be the aim. This may require from one quarter to three quarters of a gallon of material according to the length of the fleece and the type of sheep involved. The main disadvantage of jetting is that sheep have to be treated individually. This has led to attempts to develop other forms of mass treatment. So long as DDT and BHC were the only chemicals available, mass treatments gave very variable results. Fogging, whereby the insecticide is applied in oil, and light showering in spray dips, while giving some measure of control, proved to be inferior to jetting (Shanahan, 1951). Fresh interest was shown in these methods of application, when, as already mentioned, du Toit & Fiedler (1954) demonstrated the capacity of certain chemicals to diffuse down the wool fibre and to persist on the fleece. Fogging has been superseded recently by various devices which apply high concentrations of insecticides as emulsions to the surface of the fleece without penetration. This has been termed "tip spraying" or surface spraying.

Stones et al. (1954) reported protection of sheep lightly sprayed with dieldrin (0.15%) as equivalent to that obtained by dipping, when assessed by the larval implant technique. On the other hand, Wright et al. (1957), using the same test method, concluded that tip spraying was inferior to jetting irrespective of the insecticide and the concentration used. As a result of field tests, Graham (1957a, b) considered that to obtain satisfactory results it was necessary to apply from two to four times the weight of insecticide by tip spraying as by jetting. Sinclair (1959) found that when the same weight of dieldrin (0.5g.) was applied to sheep by jetting over the back and crutch area as by an all-over tip spray, only 0.6% of crutch strikes occurred in the former group, compared with 6.0% in the latter.

The evidence available, therefore, seems to indicate that jetting should be the method of choice when crutch strikes are likely to occur and fly conditions are severe. The advantages of tip spraying, namely ease and speed, could be outweighed by the subsequent labour involved in treating strikes which may not have occurred had the sheep been treated efficiently.

DISCUSSION

The sheep farmer now has at his disposal numerous effective measures for preventing fly strike. The Mules operation, in those countries where it is applicable, is undoubtedly the answer to the control of crutch strike. It is permanent and effective. Elsewhere, and for the prevention of body and poll strike, chemicals must be used. In recent years the farmer has been confronted with an array of new insecticides and new devices with which to apply them. Little attention has been paid to their intelligent use, resulting sometimes in a waste of time and materials. It is, of course, almost impossible to lay down a precise set of instructions for a problem such as fly strike, varying as it does, from place to place and time to time; more emphasis should, however, be placed by extension officers on the simple principle of adequate application of adequate concentrations at the correct time in relation to the expected period of fly attack, based on a sound knowledge of local conditions.

The search for new chemicals continues, stimulated by the appearance of flies resistant to the chlorinated hydrocarbons. At the moment most promise appears to be in the group of phosphorus insecticides. Whether sheep blowfly resistance will follow the same pattern as housefly resistance, so that, in time, these compounds are also ineffective, remains to be seen. Should this occur and other groups of active compounds not be available, the future use of chemicals in some countries at least, would appear to be limited.

ADDENDUM

Since the review was submitted for publication, S. African workers (Stampa et al., 1958) have reviewed the blowfly problem in that country. They have concluded that the protection afforded by dieldrin, aldrin and diazinon showed no significant differences under equal conditions, but that BHC was inferior. When discussing the biological conditions which may affect methods used in protecting sheep they consider that the incidence of flies exercises the most significant influences on the duration of protection afforded by any insecticide.

SELECTED REFERENCES

ANON. (1955). Rep. Commonw. sci. industr. Res. Aust., 1955.
ANON. (1957). Ann. Rep. Qd Dep. Agric. Stk, 1956-57 (p. 76).
ANON. (1958). Ann. Rep. Qd Dep. Agric. Stk, 1957-58 (p. 70).
CRAGG, J. B. (1945). Nature, Lond. 155, 394.
CRAGG, J. B. (1946). Ann. appl. Biol. 33, 127.
CRAGG, J. B. (1947). Vet. J. 103, 117.
DOWNING, W., HARBOUR, H.E. & STONES, L.C. (1952). Vet. Rec. 64, 787.
FIEDLER, O.G.H. & du TOIT, R. (1956). Onderstepoort J. vet. Res. 27, 77.
GRAHAM, N. P. H. (1954). Aust. vet. J. 30, 121.

GRAHAM, N. P. H. (1957a). Aust. vet. J. 33, 114.
GRAHAM, N. P. H. (1957b). Aust. vet. J. 33, 137.
GRAHAM, O. H. & GAINES, W. E. (1948). J. econ. Ent. 41, 521.
HARBOUR, H. E. & WATT, J. A. (1945). Vet. Rec. 57, 685.
HARRISON, I. R. & JOHNSON, C. A. (1958). Nature, Lond. 181, 1267.
HOBSON, R. P. (1936). Ann. appl. Biol. 23, 845.
HUGHES, L. E., JENKINS, J. R. W. & JONES, J. M. (1946). Vet. Rec. 58, 251.
HUGHES, L. E., POLLARD, E. P., FIELD, H. I. & JONES, J. M. (1947). Vet. J. 103, 265.
JOHNSTONE, I. L. (1951). Aust. vet. J. 27, 53.
JOHNSTONE, I. L. & SCOTT, M. T. (1951). Aust. vet. J. 27, 79.
JOHNSTONE, I. L. & SOUTHCOTT, W. H. (1954). Aust. vet. J. 30, 139.
JOINT BLOWFLY CONTROL COMMITTEE — Interim recommendations (1950). Agric. Gaz. N. S. W. 61, 557.
LENNOX, F. G. (1941). J. Coun. sci. industr. Res. Aust. 14, 77.
LENNOX, F. G. & HALL, D. L. (1940). J. Coun. sci. industr. Res. Aust. 13, 65.
LIPSON, M. & HOPE, R. J. (1955). Nature, Lond. 175, 599.
LYLE-STEWART, W. (1946). J. Minist. Agric. 53, 178.
McGREGOR, W. S. & MILLER, W. O. (1958). "Down to Earth" 14, 7. Publ'd by the Dow Chemical Co.
MacLEOD, J. (1937). Parasitology 29, 526.
MacLEOD, J. (1938). Bull. ent. Res. 29, 149.
MARQUARDT, W. C. & HAWKINS, W. W. (1958). J. Amer. vet. Med. Ass. 132, 429.
MÖNNIG, H. O. (1943). Onderstepoort J. vet. Sci. 18, 73.
MOULE, G. R., McFARLANE, B. D., YOUNG, R. B. & DERBYSHIRE, D. S. (1957). Aust. vet. J. 33, 15.
MOULE, G. R., YOUNG, R. B. & McFARLANE, B. D. (1955). Qd J. agric. Sci. 12, 101.
RICHES, J. H. & O'SULLIVAN, P. J. (1955). Aust. vet. J. 31, 258.
RICHES, J. H. & O'SULLIVAN, P. J. (1957). Aust. vet. J. 33, 34.
SHANAHAN, G. J. (1946). Agric. Gaz. N. S. W. 57, 546.
SHANAHAN, G. J. (1948). Agric. Gaz. N. S. W. 59, 98.
SHANAHAN, G. J. (1951). Agric. Gaz. N. S. W. 62, 425.
SHANAHAN, G. J. (1953). Agric. Gaz. N. S. W. 64, 328.
SHANAHAN, G. J. (1958a). Nature, Lond. 181, 860.
SHANAHAN, G. J. (1958b). J. Aust. Inst. agric. Sci. 24, 157.
SHANAHAN, G. J. & MORLEY, F. H. W. (1947). Agric. Gaz. N. S. W. 58, 660.
SHANAHAN, G. J. & MORLEY, F. H. W. (1948a). Agric. Gaz. N. S. W. 59, 263.
SHANAHAN, G. J. & MORLEY, F. H. W. (1948b). Agric. Gaz. N. S. W. 59, 271.
SINCLAIR, A. N. (1959). Aust. vet. J. (in the press).
SKERMAN, K. D. & PRYOR, W. J. (1957). Aust. vet. J. 33, 230.
STAMP, J. T., WATT, J. A. & BEATTIE, I. S. (1948). Vet. Rec. 60, 335.
STAMPA, S., FIEDLER, O. G. H. & du TOIT, R. (1958). Onderstepoort J. vet. Res. 27, 549.
STONES, L. C. (1950). Bull. ent. Res. 41, 503.
STONES, L. C., WOOD, J. C. & HART, C. B. (1954). Vet. Rec. 66, 183.
du TOIT, R. & FIEDLER, O. G. H. (1952). Onderstepoort J. vet. Res. 25, No. 4. p. 53.
du TOIT, R. & FIEDLER, O. G. H. (1953a). Onderstepoort J. vet. Res. 26, 65.

du TOIT, R. & FIEDLER, O.G.H. (1953b). Onderstepoort J. vet. Res. 26, 221.
du TOIT, R. & FIEDLER, O.G.H. (1954). Onderstepoort J. vet. Res. 26, 405.
du TOIT, R. & GOOSEN, P. J. (1949). Onderstepoort J. vet. Sci. 22, 285.
WATERHOUSE, D. F. (1944). J. Coun. sci. industr. Res. 17, 1.
WATERHOUSE, D. F. & SCOTT, M. T. (1950). Aust. J. agric. Res. 1, 440.
WRIGHT, P., PAYNE, K. & SHANAHAN, G. J. (1957). Aust. vet. J. 33, 227.

The Normal Bovine Uterus
Physiology, Histology and Bacteriology

BY

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Introduction. The main reason for this study of the normal bovine uterus is to facilitate understanding of the changes which it undergoes in disease. As uterine disease affects fertility, the study of the empty or resting uterus appears to be more urgent than that of the pregnant uterus, and as disease affects the inner tissue layers, a brief account of the neuromuscular physiology of the non-pregnant uterus precedes a discussion, in greater detail, on the endometrial histology and bacteriology. Only cursory reference is made to the pregnant condition.

Neuromuscular Physiology. This section is based on a series of experiments by N. L. VanDemark of Illinois, usually in collaboration with R. L. Hays. These workers, having read the report of John Hammond of Cambridge that milk gushed from the udder of the mare during mating, demonstrated (Hays & VanDemark, 1951) that "massage" of the external os of the cervix increased intramammary pressure by nearly 50% in 90% of their experimental cows at all stages of the oestrous cycle, and deduced that cervical massage caused the release of pitocin by the pituitary. Next, measurements by balloon and kymograph (VanDemark & Hays, 1951) showed the normal uterine contractions to be maximal around ovulation and minimal at mid-cycle; (thus parelleling observations on human subjects by pneumomanometry, e.g. Palmer, 1948). Strong contractions typical of oestrus were reproducible at any stage either by cervical massage, or by the injection of pitocin; although experimentally adrenaline inhibited the effect of the pitocin. (Similarly fright — ? endogenous adrenaline — would inhibit the effect of pitocin or massage.) Cupps & Asdell (1944) had already shown that pitocin caused strong contraction in excised uterine muscle of the cow at any stage of the oestrous cycle, but they had supposed that this would not occur in dioestrus in the intact animal owing to the masking effect of the corpus luteum. Hays & VanDemark also found that contractions were very strong during the act of service. This could be caused by oestrogen enhancing the effect of the pitocin released on stimulation of the cervix. Jordan (1952) demonstrated this effect of oestrogen while studying the effect of drugs on the puerperal uterus. VanDemark & Moeller (1951) detected spermatozoa at the abdominal ostium of the oviduct within 2–5 min. of deposition, even of dead spermatozoa, in the cervix. VanDemark & Hays (1952) analysed the additional increase in contractions which occurs during heat on the approach of the bull; four successive increases were noted, culminating at the time of ejaculation in a tetanic spasm of

the uterus lasting 1 min. which, it was concluded, was largely responsible for the rapid transport of spermatozoa from the cervix to the upper oviduct. This work was repeated by Döcke in Germany (1958), who claimed to measure the extent of the stimulus by an "activity index" which he evaluated at 30 for natural service but only 18 for artificial insemination (A. I.) – normal resting oestrous level was 9. Hays & VanDemark (1953) then showed that when blood was added to Tyrode solution perfusing the excised cow's uterus, this contracted only if the blood had been drawn after stimulation of the cervix and, presumably, contained pitocin. When pitocin was added to the perfusion fluid (VanDemark, 1953), spermatozoa placed half-way up the cervix reached the oviduct ampulla in 5 min.; when pitocin was not added, spermatozoa were not transported. Addition of adrenaline to the perfusion fluid inhibited the pitocin effect — recalling the result of the in vivo experiment in 1951. VanDemark therefore pointed out that conception rates might well be reduced in A. I., either by rough handling and fright or by insufficient stimulation of the cervix by the pipette. Dauzier (1953) showed that in the sheep spermatozoa took 9 hours to reach the ampulla, achieving this entirely through their own motility. This result has been confirmed by four other teams of workers although two teams have claimed rapid ascent in 6–20 min. as for the cow.

VanDemark & Hays (1955) gave further details of the behaviour of the excised and perfused uterus when inseminated at various stages. Only on the first to third days of the cycle did spermatozoa reach the ampulla in 5–20 min., unless pitocin was given. Pitocin improved the speed of transport in both stages of the cycle. Fitzpatrick (1951, 1957) showed that pitocin inhibits the cervix while stimulating the corpus, a finding in keeping with VanDemark's results, as a closed cervix would ensure that spermatozoa were transported only in an upward direction.

In view of Hays & VanDemark's (1951) finding that cervical stimulation could produce "oestrous" contractions at any stage of the cycle, Rowson (1955) considered that transplanted ova introduced through the cervix might be carried up by contractions induced, even on the fourth day, into the unsuitable environment of the oviduct. Oil globules were "inseminated" at known stages of the oestrous cycle. During heat the globules took 40 min. to reach the ampulla — much longer than the spermatozoa used by VanDemark. However, the administration of pitocin brought about their arrival in the ampulla within 2 min. and they ascended also on the second and third days (confirming the results of VanDemark & Hays, 1955). However, despite added pitocin, no material ever entered the tubes at mid-cycle (as Rowson expected from his earlier finding, 1951, that progesterone accelerated the movement of ova down the tubes). Rowson stated that VanDemark had claimed tubal ascent of spermatozoa at mid-cycle in the excised uterus, but the actual wording is that pitocin "somewhat improved" results from a condition of "no transport". This may imply only ascent from the cervix to the tip of the uterine horn. The marked differences in transport time observed by Rowson in the oestrous uterus, depending on the addition or omission of pitocin, suggest that his "insemination" technique may not have properly simulated natural service in stimulating the sensory endings in the external os, just as VanDemark (1953) had suggested sometimes might be the case in "too gentle"

insemination.

Veelken (1955) inseminated 68 cows mostly in dioestrus, with dead or live spermatozoa and slaughtered them 20–165 min. later. He found that after 45–55 min. 5 out of 6 had some spermatozoa in the tubes. Living spermatozoa took 45 and dead 55 min. to reach the tubes, confirming the hypothesis that the main mechanism of transport is the uterine contractions induced by pitocin. In contrast to Rowson, Veelken went even further perhaps than VanDemark, in establishing the entry of spermatozoa into the oviducts in dioestrus. This presumably resulted from more thorough cervical stimulation than that carried out by Rowson. Skjerven (1954) injected radio-opaque medium into the uterus of cows at various cycle stages and slaughtered them within 24 hours. Results (confirmed by VanDemark & Hays, 1955) were that muscular contraction during the first to third days of the cycle caused rapid movement of medium into the oviducts and abdomen, though at other stages the amount instilled was recoverable from the uterus. Skjerven hence pointed out that disinfectant uterine irrigation during the first to third days might not only be relatively ineffective against organisms on the endometrium, but that the solution might wash the zygote out of the oviduct into the abdomen.

Histology. The cyclic changes concern chiefly the endometrium, which consists of a surface epithelium overlying a connective tissue stroma containing glands, blood vessels and mobile cells. The cyclic changes of each component are considered separately. The following reports have been used:-

America – Kingman (1944); Asdell et al. (1949, 17 heifers); Weber et al. (1948, 22 heifers); Roark & Herman (1950); Foley & Reece (1954, 36 heifers and 17 cows).
Germany – Vollmerhaus (1957, 80 cows and heifers).
Hungary – Széky & Dózsa (1955, 1956, 114 biopsies in cows and heifers).
Italy – Tagliavini (1934); Addis (1935).
Norway – Skjerven (1956, biopsies in 106 cows and 16 heifers).
Great Britain – Hammond (1927, 6 heifers); Dawson (1950, 19 cows); Azizuddin (1954).

The Surface Epithelium. Széky & Dózsa claim that up to the age of 18 months this epithelium consists of a single layer of columnar cells, and that during the next 6 months a second, lower layer with larger nuclei develops (and still others during dioestrus). No such sequence has been noticed by other workers, though Kingman declared that the epithelium had multiple layers, possibly only briefly during dioestrus, thus confirming Tagliavini's report. Foley & Reece, who studied both cows and heifers, insist on a single layer. They point out that an illusion of stratification can be produced by section at an acute instead of a right angle to the plane of the surface. Vollmerhaus who studied cows and Hammond who studied only heifers, also report a columnar epithelium consisting of a single layer. Weber et al. and Asdell et al. working on heifers only, and Roark & Herman, all describe this epithelium as "pseudostratified" and show that an appearance of stratification can be produced, possibly in the way suggested by Foley & Reece. Széky & Dózsa's results may receive

confirmation when other workers investigate both cows and heifers for this difference. According to Weber et al., cyclic erosion of the surface epithelium, which they note is occasionally ciliated, occurs on about the third day of the cycle, especially in heifers. There is extravasation of blood, and the erosion is preceded by a discrete necrosis during pro-oestrus. This may correspond with a de-epithelization observed on about the 17th day by Tagliavini, who however reported no post-oestrual erosion. None of the other workers refers to either of these processes. Vollmerhaus, and Széky & Dózsa, attribute a secretory function to the surface epithelium, but whereas Vollmerhaus considers that secretion occurs from the twenty-first day to the third day, Széky & Dózsa limit it to dioestrus.

Observations on the cyclic changes in the surface epithelium are scanty. During heat (the first day), according to five investigation teams, the cells are tall with the nuclei basal and large; they may be vacuolated. During met-oestrus, Asdell et al. observed that the cells were shortest on the third day. According to Dawson, the nuclei on the fourth day are not basal but occur at varying levels. By the fifth day Weber et al. observed distortion of the regular structure with many mitotic figures, and four workers agree that during full dioestrus the cells are tall again with basal nuclei, which may be hard to see (Roark & Herman), — this is confirmed by Vollmerhaus' finding that nuclear volume is at its lowest from the seventh to eleventh days. Dziuk et al. (1958) found minimal loss of surface epithelium when stored in fixative during this period. During early pro-oestrus, Tagliavini, Dawson, and Széky & Dózsa agree that the cells are at first reduced in height and become cuboidal, with basal nuclei. Weber et al., however, report tall cells with elongate nuclei at pro-oestrus. Dawson agrees that by the 20th day the height has again increased and the nuclei have left the base.

The Sub-Epithelial Connective Tissue (Stroma). Hammond, Weber et al., Széky & Dózsa, and Skjerven, all recognize a stratum compactum ("lamina propria" of Weber) just below the surface epithelium during heat, with more diffuse strata spongiosa beneath. Vollmerhaus considers that these two layers can be differentiated only during dioestrus (the fifth to fifteenth days). The stroma consists essentially of white fibrous tissue (Weber) with the precursors of the plasma cells and histiocytes which it forms in tissue culture (Széky & Dózsa). There is general agreement on the cyclic appearance of oedema. Vollmerhaus, Kingman, Asdell et al., and Skjerven, consider oedema to be greatest during oestrus, and so by implication do Roark & Herman, who saw "more stroma" from the second day onwards. Weber et al., however, described "lacunae" at heat with progress into full oedema only on the third day, and Hammond's results agree. Weber considered the oedema to be confined to the spongiosa, but Skjerven observed it in the compacta. Asdell et al., experimenting in castrated animals, obtained evidence that the oedema was caused by circulating oestrogen. It is difficult to accept Széky & Dózsa's unconfirmed claim that oedema begins at oestrus, is greatest at dioestrus and disappears only during pro-oestrus (Széky & Dózsa also claimed to have demonstrated marked metabolic activity, with the presence of enzymes). Vollmerhaus measured the lacunae and found them smallest at dioestrus,

the 12th day, their area being only 29% of that during oestrus. Hammond did not observe oedema at dioestrus.

The Endometrial Glands. Only Vollmerhaus observed that each gland consists of a duct (sometimes composed in part of ciliated cells – Deininger, Derivaux) traversing the compacta and spongiosa, into which drain some 30 tubular glands which originate in the spongiosa. The cyclic variations in size and development are considerable. Széky & Dózsa considered that development was least during pro-oestrus, or more rarely, during heat. Vollmerhaus measured the gland volume compared with the total mucosal volume, and found the minimum, 4.5%, during heat. Azizuddin thought size decreased rapidly during the second and third days, Asdell placed the first signs of growth on the third day, and Hammond's heifers showed least development on the second day. Five authors agree that the tubes become more coiled during dioestrus; while Roark & Herman state that coiling has begun by the third day and quote Cole (1930) that the glands grow steadily for 11 days after heat; according to Asdell coiling is greatest between the 8th and 12th days, while Vollmerhaus found coiling at its peak by the 12th day. Vollmerhaus considers the coiling has often been described, mistakenly, as an increase in the number of gland tubes, but only Azizuddin, among the authors considered here, has mentioned an increase in number, slow at first, during dioestrus, followed by a decrease during the next phase.

Regarding the extent of the secretion, which is reflected in the appearance of the lumina of the tubes, Hammond, confirmed by Roark & Herman, states that the lumen is invisible at heat. Vollmerhaus notes that the tubes are circular in cross-section at heat. By the third day Hammond still found the lumen invisible, but on the fourth, Dawson detected it. Vollmerhaus found the peak of glandular development on the 12th day – 27% of the total mucosal volume or a sixfold increase. Széky & Dózsa claim to have detected the onset of secretion by a change in the cytoplasm of the gland epithelium, which becomes granular. But Vollmerhaus thought secretion was at its peak on the fifth day, declining from the 12th day, and this confirms the finding of Asdell et al. that secretion declines from the eighth day. Hammond found the lumen wide on the eighth day as did Dawson on the 12th day, when Tagliavini observed that the ducts were dilated with secretion. Széky & Dózsa stressed that at dioestrus the glandular secretions are thread-like.

The changes in the glands after the 12th day have been studied in less detail. During regression/pro-oestrus Széky & Dózsa noted that the secretion threads were less evident and Weber found that 20% of the terminal gland ducts were necrotic. Gland size and extent of coiling ("gland number") are reduced and secretion ceases (Tagliavini). Dawson observed a reduction of gland tissue up to and including the 18th day. The lumen was still wide, confirming Hammond, who like Széky & Dózsa observed secretion products. There followed a progressive increase in glandular tissue, with narrowing of the lumen. These observations were confirmed by Széky & Dózsa's finding that glandular development was least, more often during pro-oestrus than later.

With regard to cellular changes, at heat the nuclear chromatin is more dense and the cytoplasm is homogeneous (Széky & Dózsa); the cells

grow taller, with basal nuclei. In the next phase, Skjerven found that mitosis was most rapid during the second and third days. This confirms the observations of Roark & Herman, Dawson, Széky & Dózsa, and Vollmerhaus on the general rate of glandular development, but is inconsistent with those of Hammond, Asdell et al., and Azizuddin, who found little or no development at that stage. The explanation may be an individual or breed variation. Hammond found the cells growing on the third day, and they were already tall on the second day (Tagliavini), with clear cytoplasm at the apex (confirmed by Széky & Dózsa). By the fourth day the cells were tall, with basal nuclei (Dawson). During dioestrus, only Vollmerhaus detected vacuolation in the cells, the fourth to tenth days. Hammond and Dawson agreed that the cells increased further in height; the nuclei were still basal according to Dawson, and Széky & Dózsa, but central according to Tagliavini. Széky & Dózsa observed diffuse nuclear chromatin, and granular cytoplasm, while at pro-oestrus there was general agreement (Hammond, Széky & Dózsa, Dawson, Vollmerhaus) that the cells were much reduced in height. Széky & Dózsa noted some degeneration, and Dawson a progressive increase in height again from the 19th day onwards.

Vascularization. Cows and heifers differ. In the heifer the elastic tissue of the arteriole wall is thin, and the blood supply to the caruncles poor. In the cow the elastic tissue thickens with successive pregnancies (Tagliavini), and there is a progressive increase in number and extent of coiling of the vessels surrounding the caruncles. Blood supply is poor during dioestrus, the vessels are narrow (Széky & Dózsa, Tagliavini) with thick walls, under the influence of progesterone (Széky & Dózsa), and few or no arterioles are visible above the muscle. Vascularization may begin to increase as early as the 18th day, and by the first day the superficial capillaries are distended (Dawson). Congestion increases until the third day (Hammond). At oestrus/post-oestrus extravasation takes place in two stages, first below the surface epithelium and then through it into the lumen where, in all heifers, the free blood clots and is ejected. Such a discharge was observed in only 61% of cows, mostly on the second day (Trimberger, 1941). Microscopic observations show a corresponding difference in haemorrhage. In heifers Hammond observed much sub-epithelial extravasation on the second day, at first on the caruncles and later between them, while Weber et al. described the formation of blood vesicles on the third day in and above the compacta. In the second stage there was capillary haemorrhage into the lumen between the caruncles (correlated with the erosion of surface epithelium), and also outward diapedesis, producing free blood clots in the lumen. Weber quotes Zietschmann (1922) and Zupp (1926) as describing bleeding chiefly on the caruncles. Presumably (in the light of Hammond's and Trimberger's results) the bleeding is at first mainly from the caruncles and later between them. Dawson observed congestion between, not on, the caruncles on the fourth day. Other workers, using material from cows or from both cows and heifers, observed similar but less striking extravasation. Weber's timing was confirmed by Vollmerhaus, who found conspicuous blood sinuses beneath the surface epithelium, which ruptured and discharged from the second to fourth days. Tagliavini recorded

sub-epithelial haemorrhage and a slight oozing at the surface during heat. He quoted Krupski (1917) that this haemorrhage might be greater in the horn on the ovulating side. Dawson found "internal" haemorrhage from the distended capillaries in cows during heat, as did Skjerven, who observed diapedesis between the surface epithelial cells, and increasingly on the second and third days. Dawson confirmed Krupski's work in one animal; post-oestrous congestion was much greater in the horn of the ovulating side. Széky & Dózsa considered free blood beneath the epithelium to be a pathological sign, but their photograph of a normal oestrous section shows many free erythrocytes. On the 21st day Dawson observed a clear or milky lymph-like fluid lying free on the surface in two of three cows, as did Hammond in a heifer. No observer has reported this during the phase of glandular secretion. Foley & Reece remark that there is neither evidence nor agreement on the function of "uterine milk".

The Mobile Stroma Cells. Leucocytes: only the polymorphs are reported to show cyclic variations. They are conspicuous during pro-oestrus in the stroma and between the cells of the surface epithelium, especially around the gland ducts (Roark & Herman, Skjerven). Tagliavini, and Széky & Dózsa, record infiltration during heat. Weber et al., however, differ from other authors, perhaps because all their material was from heifers. Weber observed few leucocytes during pro-oestrus, infiltration into the compacta beginning only on the second day; by the third day (when the epithelium was eroded) polymorphs were streaming into the lumen; infiltration, mainly of lymphocytes, continued on the fifth day, and had ceased by the seventh day. Murphey (cited by Roark & Herman) supports these findings. He observed infiltration to begin in pro-oestrus on the nineteenth day and last until the eighth day. Tagliavini records that during dioestrus (presumably the fourth to eighth days) polymorphs were still numerous in the mucosa and free in the lumen. Weber found eosinophiles to be rare but they were not uncommon in Skjerven's material. Again this is possibly an age difference.

Mast Cells: these were first observed below the surface epithelium by Tagliavini, who considered that they appeared only during dioestrus. Addis (1935) noticed that mast cells (which he termed histiocytes) contained conspicuous granules and seemed to occur only in parous animals. Széky & Dózsa confirmed their presence in the compacta. The work of Skjerven shed fresh light; mast cells were commonest in the compacta, and the number varied irrespective of the stage of the cycle. Addis' work on the content of metachromatic granules and on the greater incidence in cows than in heifers was confirmed. Skjerven showed that a very few mast cells occurred regularly in heifers and suggested that they might be concerned in the formation of heparin. Other granules that were present were positive in the periodic acid–Schiff reaction for glycogen.

Plasma Cells: a few were noted by Tagliavini, and by Széky & Dózsa, in the compacta. Weber et al. found numerous plasma cells in only two of their 22 heifers. Skjerven's more intensive work revealed that they were in general rare, except in old cows, and increased steadily with age, and this observation was confirmed by de Bois & van den Akker (1957) in biopsies. Their presence is interpreted as a local immune reaction to repeated post-puerperal infection.

Macrophages (Histiocytes): a few were observed in the compacta, only during pro-oestrus, by Weber et al., and irrespective of stage by Széky & Dózsa. Skjerven found small numbers at all stages in various situations. They contained pigment, possibly from ingested erythrocytes. Széky & Dózsa noted a significant proportion of monocytes during dioestrus.

Lymph Nodes: Skjerven found some lymph nodes in the endometrium of 13 out of 19 supposedly normal cattle; they were, however, frequent in cows with purulent endometritis associated with C. pyogenes and he considered that they are probably always caused in this way. Simon & McNutt (1957) confirmed this view. Tagliavini considered that lymph nodes were normally present but his material probably resembled Skjerven's. It is noteworthy that lymph nodes (and plasma cells) were noted by Weber et al. in two of their 22 heifers. As lymph nodes and plasma cells are associated with endometritis, it seems likely that these two heifers were affected with it.

The Pregnant Uterus. A full account is outside the scope of this paper but a few points are mentioned to compare pregnancy with dioestrus. Kingman and Dawson agreed that the glands were more distended with secretion in early pregnancy than in dioestrus. Kingman reported the intercaruncular epithelium as thin and consisting of a single layer. Hatch (1941) observed from the 21st day a partial destruction of the epithelium, which survived only at the mouths of the gland ducts, and Dawson confirmed this. But Wrenn et al. (1954) suggested that Hatch had observed artefacts.

Conclusions. In the light of existing knowledge, only a very broad correlation can be made of the changes characterizing each cycle stage.

At oestrus there is (a) considerable vascular congestion which may later reach a peak; (b) oedema in the stromal zone (proved to be an effect of oestrogen); (c) development of the gland epithelium and concentration of leucocytes near the surface.

During the second to fourth days the glands develop rapidly and begin to secrete, vascularization and infiltration of leucocytes reach their peak and oedema disappears.

Glandular secretion is greatest from the fifth to twelfth days and becomes progressively slower for several days after. The mucosa, however, perhaps surprisingly, appears relatively avascular and dense. The surface epithelium may grow in height.

The 12th to 21st days may be divided into regression and pro-oestrous phases. During the regression phase the glands uncoil, shorten and become less active: the surface epithelium shows similar phenomena and the stroma may become even more dense and less vascular. In the pro-oestrous phase, vascularization, stromal oedema, infiltration of leucocytes and glandular development begin, though there may be wide individual variation. Some of these phenomena are due to oestrogen, and the glandular development is, of course, an effect of progesterone.

Hansel & Asdell (1952) claimed that post-oestrous bleeding was due to the withdrawal of oestrogen. The concentration of leucocytes at and immediately after oestrus may be a defence against infection, since it is only then that the endometrium is exposed to bacterial infection from the exterior via the vagina and cervix. However, it does not follow that

"oestrogens are disinfectant"; this effect appears to depend on the succession of a luteal phase. Continued stimulation by oestrogen is often associated with a septic uterus (e.g. Weinstein et al., 1937; Dawson, 1958; Terpstra, 1956).

Disagreements between investigators can be accounted for, largely, by the physiological differences between the cow and the heifer. The position would be clear had these workers realized the importance of separating the results from cows and from heifers. Not so explicable is Széky & Dózsa's description of stromal oedema beginning at heat and reaching its peak only at mid-cycle. This is against all the other evidence. The differences between the cow and the heifer are concerned mainly with the vascular changes around heat. Extravasation occurs in all or nearly all heifers, but only in some cows and then on a small scale. The increased incidence of mast cells with age in cows suggests that their main function here is unlikely to be the production of the anti-coagulant heparin, despite the results of five independent teams of workers (quoted by Skjerven) on the function of these cells at other sites. Leucocytes may infiltrate later and on a larger scale in the heifer. The low incidence of plasma cells in heifers, identified as a local immune reaction, fits the picture that the maiden and especially pre-cyclic uterus is aseptic and becomes exposed to bacterial invasion only during oestrus and service. The extravasation is explicable as a flushing device to reinforce the direct antibacterial action of the leucocytes. The mature cow has learned to live with certain bacteria in utero, and immunity mechanisms, shown by the presence of plasma cells and lymph nodes, have become established. The flushing mechanism then becomes less important and is modified to play a smaller role. The possible difference between the cow and the heifer in the structure of the epithelium may be due to erosion during post-oestrual flushing in the heifer.

Future work should repeat Széky & Dózsa's study of animals of different ages; groups of heifers under 18 and over 24 months should be investigated, particularly as regards surface epithelium, lymph follicles and plasma cells, and compared with extensive series of normal and infertile cows, classified into pregnancy age-groups. The last nine days of the cycle have been very imperfectly studied, and the regressive and proliferative phases are barely known. Only a small number of cows studied by Dawson have furnished information on the behaviour of the glands and blood supply during this period.

Histochemical Reactions. The main study on histochemical reactions in the endometrium is by Skjerven (1953, 1956), who performed an average of more than two biopsies in each of 106 normal non-pregnant cows and 16 heifers. All other workers used post-mortem material. Weeth & Herman (1952) used ten non-pregnant and eight pregnant cows, while Foley & Reece (1953), Foley et al. (1954) and Wrenn et al. (1954) worked respectively on 53 and 14 pregnant cows and heifers. Plebani (1953) in Italy studied the uterus in 32 cases of pyometra, which he stained for glycogen only, by the iodine method of Langhan. All other workers used techniques to reveal alkaline phosphatase; Skjerven and Foley et al. studied neutral fat and Foley et al. the

distribution of cholesterol. Both Skjerven and Wrenn et al. stained for glycogen, as did Derivaux et al. (1957).

Results did not always agree; discrepancies may be due to the American workers, in contrast to Skjerven, having relied mainly on pregnant material, or to the use of varying techniques to reveal specific substances. All save Plebani used the periodic acid–Schiff reaction for glycogen, but different reactions were used for neutral fat, and Skjerven eschewed Gomori's alkaline phosphatase method, favoured by the Americans.

Foley et al. and Wrenn et al. agree that the uterine glands have a moderate to high content of alkaline phosphatase in early pregnancy and Skjerven's findings were similar for the comparable dioestrous phase. All workers agree that the blood vessels are rich in the enzyme, Foley et al. noting that the lining layer was intensely positive. Skjerven and Foley et al. concur that the superficial connective tissue contains moderate amounts. Skjerven found cyclic variations in the content of alkaline phosphatase both in the surface epithelium and to a lesser extent in the glandular epithelium. The concentration at the surface was low at oestrus, rose during dioestrus and was declining by pro-oestrus, whereas in the glands the concentration was highest at oestrus. Skjerven found that fat showed a similar pattern in the surface layer; it was absent from the nineteenth day to the sixth day (when there is no functional corpus luteum), then appeared suddenly, to persist during the remainder of the cycle. It might, therefore, have been expected to occur in the early pregnant uterus, but Foley et al. reported little fat at that stage except in the chorion. However, Foley et al. found much cholesterol in the surface epithelium, especially between the caruncles.

Skjerven found the concentration of glycogen to vary markedly with the stage of the cycle; the variations were confined to the surface epithelium and to the connective tissue of the stratum compactum. In the surface epithelium glycogen disappears sharply at the same time as the fat returns, at the seventh day of the cycle, and remains absent until the thirteenth day; this observation was confirmed by Derivaux et al. (1957), who published an extensive series of photomicrographs. It is only between the seventh and thirteenth days that the stratum compactum is full of glycogen granules, which seem to migrate there from the surface epithelium. The blood vessels and glands always have a moderate glycogen content. Plebani quotes Frei, and Bignardi, as stating that the glycogen content of the "mucosa" is greatest in the luteal phase, which appears to tally with the content in the stratum compactum observed by Skjerven. (In endometrial sections the surface epithelium becomes detached very readily during processing. This may have occurred in some of the material of Frei and Bignardi.) Wrenn et al. state that in early pregnancy the glycogen (and alkaline phosphatase) content of the surface epithelium resembles that in pro- and met-oestrus, which, according to Skjerven, would then be moderate to low in alkaline phosphatase and high in glycogen. This contrasts with Skjerven's findings that in dioestrus (a stage perhaps more comparable with early pregnancy) there is no glycogen and a rising phosphatase content. But since Wrenn et al. themselves showed that the dioestrous corpus luteum contained much

alkaline phosphatase and the pregnancy corpus luteum none, there is need for further work, in both pregnant and non-pregnant cows.

Plebani, Wrenn et al., and Skjerven, all agree that the uterine distribution of glycogen and phosphatase is a "corpus luteum effect". But Wrenn et al. deduced that the pro- and met-oestrous ovary secretes large amounts of progesterone, because glycogen and phosphatase have a similar distribution then to that in early pregnancy; the data seem insufficient to warrant this.

Plebani pointed out that progesterone is known to "immobilize", and oestrogen to mobilize glycogen in the liver. He regarded the mucosal (stratum compactum?) glycogen as "immobilized" by progesterone from the corpus luteum, and Skjerven agrees: "the surface epithelium glycogen content may be an expression of the oestrogen level", the mobilized glycogen being considered in the process of passing into the lumen. Of Plebani's 32 specimens of uterus with pyometra, 22 contained at least normal amounts of mucosal glycogen. (This was almost certainly in the stratum compactum, as the surface epithelium seldom survives in pyometra.) Higher than normal amounts were recorded in 13 of these 22 and Plebani associates this with retention of the corpus luteum. The function of glycogen, phosphatase and fat is obscure and distribution differs widely between species.

Csapo (1956) reported a high calcium and low sodium content in the proliferative endometrium, and the converse in the secretory phase.

Bacteriology. General Survey. In Table I appear the results of bacteriological studies of the normal uterus, by nine teams of workers. There is general agreement that micrococci are the commonest organisms, averaging about 60% of all recoveries. Four teams recovered Micrococcus pyogenes var. aureus (haemolytic) as half or more of the micrococci, but Gunter et al. found only 8% to be coagulase-positive. Six teams found streptococci to be the second or third commonest genus. The streptococci were predominantly non- or alpha-haemolytic. Str. acidominimus has been identified in the vagina also by Smith & Sherman (1939) and by Francis (1941). The recovery by Gunter et al. of the diphtheroids Corynebacterium bovis and Microbacterium lacticum almost as often as micrococci is noteworthy, and unique in this series; possibly this was due to employment of thioglycollate media, which none of the other teams used. By contrast, although both Martínez and Klimmer et al. tried a variety of media under anaerobic, aerobic and raised CO_2 tension conditions, about 99% of their organisms were aerobes growing readily on ordinary media. So the results of Dawson and of Easley et al., who used only blood agar plates for most of their cultures, are not altogether invalid. Anthracoids recorded three times may be assumed to be contaminants, but all the other genera mentioned appear to belong to the normal flora.

Table I shows that the proportion of normal uteri recorded as bacteriologically sterile varies greatly. Apart from the possibility suggested by the use of thioglycollate media, there appears to be no obvious correlation between "inadequate" culturing techniques, and high percentages of "germ-free" uteri. It may be significant that the greater

TABLE I.

Bacterial Flora of the Bovine Uterus and Vagina

UTERUS

	Place Date	No. of organs studied	Staphylo-coccus	Micrococcus	Streptococcus	Other Diphtheroids	C. viridans pyogenes renale	Pseudomonas	Escherichia coli	Neisseria	Other Organisms	% Sterile
Klimmer et al.	Germany 1929	22	4.5		18 3 Str. incl. acidominimus				4.5		4.5	72
Fitch & Bishop	U.S.A. 1932	126	3.3	3.3		9.2			3.3		3.3 Alkaligenes	77.6
Mjuljberg	Russia 1937	33	("Mainly coccal forms") 15									35
Dawson	England 1950	19	28 haemolytic (aureus) 11 n-h* albus (accompany aureus).		6 n-h.			6	12	6	12 (unident. and Proteus.)	58
Easley et al.	U.S.A. 1951	148	26 Micrococcus pyogenes	23 others	10 Str. acidominimus.	4		8	"Few"	"Few"	"Few" Proteus anthracoids	39
Kampelmacher	Holland 1952	47	17, haemolytic and non-haemolytic.		2						anthracoids	80
Bratke	Germany 1952	80		6 M. citreus	"Few"		"Few"	"Few"	"Few"			less than 52
Gunter et al.	U.S.A. 1955	92	8% of Micrococci coag. positive.	55, viz. 21 aureus 23 albus 11 others.	19 1 pyogenes 1 bovis 17 faecalis	C. bovis 36 Microbact. lacticum 50	C. pyogenes 3.5		7.5 (plus 5 Aerobacter)			25 (38)
Martínez	Spain 1955	100	5 species NOT M. pyogenes. about 60		15 Str. acidominimus						anthracoids Flavobact. Bacteroides Achromobact.	17

VAGINA

Sýkora	Bohemia 1932	not stated	Staph. aureus 70 " citreus 20 " albus 30		50				40			
Weitz	England 1947	100	n-h. Staphs. about 50 * haem. Staphs. 30 (1 herd).		alpha-haem. about 90	about 30	C. pyogenes 1 renale about 40		40 (3% haem.)			
Dawson	England 1948	55	n-h. Staphs. 44 haem. Staphs. 33 (3 herds).		65 green 13 n-h. (1 herd)	4	C. pyogenes 2		44 (7% haem.)			
Makhinya	Russia 1957	not stated		16% candicans minimus parvus.	3.5 Str. vaginitidis	7 C. pseudodiph.	C. pyogenes 3 renale 13		about 30 (plus B. aromafaciens)		B. cereus Sarcina.	

The numbers under the various species of bacteria represent the percentage of organs in which the bacteria were found.

*n-h = non-haemolytic. * haem. = haemolytic.

the number of uteruses studied, the lower tends to be the proportion which are sterile. For example, Easley et al., Martínez, Gunter et al. and Bratke studied respectively 148, 100, 92 and 80 cows and recorded that in 39, 17, 25 and about 50% the uterus was sterile. Klimmer et al., Mjuljberg, and Kampelmacher studied respectively only 22, 33 and 47 organs, so their samples may be too small to be significant.

Comparison with the Vaginal Flora. The results of four independent workers have been chosen as representative. They show a remarkable agreement that, as in the normal uterus, micrococci and streptococci are the most important numerically (each recovered from 50% of vaginal samples), closely followed by Escherichia coli, about 40%, and by Corynebacterium renale, 13–40%. Corynebacterium pyogenes occurs in 1–3% of samples, and other diphtheroids such as C. pseudodiphtheriticum, somewhat more frequently. The most obvious differences from the uterus are the high incidence of C. renale and E. coli, associated respectively with the proximity of the urinary meatus and anus.

Retrospect. At this point the history of the concept "the healthy uterus is generally germ-free" may usefully be traced in some detail. Originating in human physiology, the concept did not have an immediate appeal in the veterinary sphere. Sohnle (1901) founded a "school" which maintained the contrary so far as the mare was concerned, and later various workers, including Kunst (1911) among the earliest, obtained evidence for a similar condition in the cow. Wall (1914) reported the recovery of C. pyogenes from 116 and streptococci from 61 of a series of pregnant uteruses studied. Day (1917) also supported the view that a uterine flora was normal, and recovered organisms from 42% of 43 uteruses. Wagner (1927) recovered organisms from the uterus in 61% of pregnant cows.

Carpenter (1920) in New York State obtained negative cultures from the uterus in 55 out of 56 maiden heifers (one yielded streptococci), and Knorpp (1928) in Munich recorded a series of negative cultures from normal cows. But it was Klimmer et al. (1929), and particularly Haupt (1930), who first contended strongly that the normal uterus is sterile. Haupt admitted that organisms had been found repeatedly, but claimed they had been introduced as contaminants in abnormal semen. Mjuljberg concluded that the endometrial mucosa was uniformly sterile after he had obtained negative cultures from the broth used to rinse out the lumen of 33 organs. Any organisms which he found were from the thickness of the wall. During the 1930's and 40's Haupt's view obtained some acceptance. Clark & Stevenson in Canada used Mjuljberg's rinsing technique in their study of 13 non-pregnant cows and also cultured the foetal fluids from 9 pregnant ones; all their cultures were negative. As late as 1952, Kampelmacher, after obtaining bacteria from 20% of the organs he studied (Klimmer et al. 28%, Fitch & Bishop 22.4%), declared that "the healthy uterus is normally germ-free".

The opposite view persisted. In France Charpentier (1949) and Craplet (1952) were convinced that there was a normal uterine flora, but did not publish evidence. The Czech, Sýkora (1932) found organisms in about half the normal uteruses studied. Azizuddin (1947) isolated organisms from nearly half of 40 uteruses. Detailed recent work, with more representative samples of material, (see Table I) seems to establish

that a flora is in fact present in 60–80% of normal cow uteruses at certain times. Carpenter's observation on the low incidence of organisms in the uterus of maiden heifers may support Haupt's view that the flora derives from semen, but surely not from abnormal semen. In this connexion Bratke (1952) has drawn attention to the 40% incidence of C.pyogenes found by Hancock & Kelly (1948) in semen which gave normal results at insemination.

Assessment of Results in relation to Techniques. Cultural methods and media have been discussed. Methods of obtaining an inoculum included the rinsing technique used by Mjuljberg and by Clark & Stevenson. In theory this should ensure that every organism on the mucosa is given a chance to multiply on the culture plate, but in practice no recoveries have resulted (though the method has been applied only to 46 organs). The simpler method by which the mucosa is stroked with a platinum loop, practised by Klimmer et al., Fitch & Bishop, Azizuddin, Dawson, and (in normal organs) Kampelmacher, consistently yielded organisms in 20–40% of post-mortem specimens. These specimens represented mainly inter-oestrous phases of the cycle, but with an average number of uteruses investigated per worker as low as 40, the representation even of these phases may be inadequate. (Fitch & Bishop did not use blood-enriched media, a serious omission.)

All other investigators studied the living animal, taking samples during heat and at no other time. Bratke used a simple endocervical swab, while Martinez sucked a sample of mucus by pipette from the middle of the cervical canal. Easley et al. inserted a pipette into the uterus through a tubular speculum placed in the cervical canal. This development foreshadowed the apparatus described by Frank & Bryner (1952) in which a tube within the speculum (6 mm. in diameter) can be used either to pass a sterile swab through a rubber diaphragm, or as a receptable into which to suck mucus. This was the method adopted by Gunter et al. Kampelmacher carried the principle further still, evolving a technique by which a cervical speculum 7 mm. in diameter is inserted as far as it will go, and a biopsy instrument of Nielsen type is passed through it into the uterus. A portion of endometrium is withdrawn and used for inoculating media, but only infertile cattle were sampled in this manner. Disinfection of the external os and anterior vagina markedly reduced the number of positive cultures obtained and was thus essential. Strikwerda (1956) improved the design of the instrument, so that the speculum could be omitted; he considered that his modification reduced contamination, as he obtained 10 pure cultures in samples from 48 cows, whereas Kampelmacher obtained none from 82 cows. Tincture of iodine was used to disinfect the os and anterior vagina. With the same technique, de Bois & van den Akker (1957) obtained 10.2% bacteria-positive samples, all in pure culture, among 137 cows presented for insemination.

The low proportion of sterile samples found by Martínez, Gunter et al. and Easley et al. may be due either to their large and therefore more representative sample of cows, or to the fact that their studies were confined to cows in oestrus. Mjuljberg considered that bacteria increased during dioestrus and more recently it was believed widely that the oestrous uterus is "disinfectant". But Weinstein et al. (1937) showed, in

mice, that vaginal organisms traverse the open cervix and enter the uterus during heat, and the similarity between the cocci and diphtheroids (not C. renale) found in the bovine uterus and vagina suggests that a similar mechanism operates in the cow, (in addition to the introduction of bacteria by semen). Weinstein et al. found that the uterine flora in the mouse is richest in early post-oestrus. Now the rhythmic alternation between oestrous and dioestrous (secretory) phases has a powerful effect in eliminating pathogenic bacteria from the uterus, these flourish when either uterine phase becomes chronic (nymphomania and retained corpus luteum respectively). The same mechanism may affect the normal flora causing a peak in the first few days after oestrus and a trough 10–14 days later. This coincides with the lower incidence of bacteria reported in dioestrous material. It could therefore be expected that a richer flora might be found during pregnancy, when the luteal phase supervenes and becomes continuous, than during dioestrus. But unfortunately it is difficult to make a useful assessment in this condition, since a mid-stage pregnancy can continue for some time in the presence of even frankly pathogenic organisms such as Brucella and C. pyogenes.

Conclusions. It was established by 1950 that 20–40% of uteruses contained bacteria. Micrococci and streptococci were the commonest with a few Escherichia coli. Dawson recorded haemolytic properties in some of the micrococci and the occurrence of Neisseria, Pseudomonas and Proteus. Between 1951 and 1955, Easley et al., Gunter et al., and Bratke confirmed the regular occurrence of some Neisseria, Pseudomonas and Proteus, and the first two teams reported the haemolytic properties of some micrococci. Gunter et al. showed some of these to be coagulase-positive and found, as well as a few C. pyogenes, a high incidence of other diphtheroids (perhaps by using thioglycollate media). Bratke (1954) claimed to have identified a new corynebacterium, C. viridans.

There is much scope for future work. Carpenter's study of the uterus of the maiden heifer should be repeated, and would throw light on the role of semen as suggested by Haupt. Experimental insemination with bacterium-free semen should be possible. Many more dioestrous uteruses must be examined post-mortem, comparable with the numbers of those studied in vivo during heat, to establish the varying incidence of sterile organs in heat and in dioestrus. It is worthy of note that Skjerven (1956) apparently took biopsy samples during dioestrus without harming the animals. Opinions differ on the influence of hormones on uterine bacteria. More work is required on the bacteriology of the healthy pregnant uterus, using organs from herds where agglutination testing is carried out, records are kept, and where the control animals in each batch are maintained to full term. Conditions under which the normal flora may become pathogenic should be investigated.

REFERENCES

ADDIS, M. (1935). Nuova Vet. 13, 406.
ASDELL, S.A., de ALBA, J. & ROBERTS, S.J. (1949). Cornell Vet. 39, 389.
AZIZUDDIN, I. M. (1947). Personal communication.

AZIZUDDIN, I. M. (1954). Indian vet. J. 30, 376.
de BOIS, C. H. W. & van den AKKER, S. (1957). Tijdschr. Diergeneesk. 82, 951.
BRATKE, E. (1952). Berl. Münch. tierärztl. Wschr. 65, 247.
BRATKE, E. (1954). Fortpfl. ZuchtHyg. Haustierbesamung 4, 64.
CARPENTER, C. M. (1920). Rep. N. Y. State Vet. Coll. 193.
CHARPENTIER, J. -C. (1949). Thesis, Paris (Alfort).
CLARK, W. A. & STEVENSON, W. G. (1949). Canad. J. comp. Med. 13, 92.
COLE, H. H. (1930). Amer. J. Anat. 46, 261.
CRAPLET, C. (1952). Reproduction normale et pathologique des bovins. pp. xxi + 260 Paris: Vigot Frères.
CSAPO, A. (1956). Amer. J. Anat. 98, 273.
CUPPS, P. T. & ASDELL, S. A. (1944). J. Anim. Sci. 3, 351.
DAUZIER, L. (1953). C. R. Soc. Biol., Paris 147, 1556.
DAWSON, F. L. M. (1949). Diss., Cambridge.
DAWSON, F. L. M. (1950). J. agric. Sci. 40, 150.
DAWSON, F. L. M. (1958). Brit. vet. J. 114, 96.
DAY, L. E. (1917). Rep. N. Y. State Vet. Coll. p. 80.
DEININGER, G. (1957). Inaug. Diss., Munich.
DERIVAUX, J., BIENFET, V. & JOARLETTE, P. (1957). Ann. Méd. vét. 101, 527.
DÖCKE, F. (1958). Zuchthyg. FortpflStörung. u. Besamung 2, 266.
DZIUK, P. J., DONKER, J. D., NICHOLS, J. R. & PETERSEN, W. E. (1958). Tech. Bull. Univ. Minn. agric. Exp. Sta. No. 222.
EASLEY, G. T., LEONARD, R. H. & TROTTER, D. M. (1951). N. Amer. Vet. 32, 258.
FITCH, C. P. & BISHOP, L. M. (1932). Cornell Vet. 22, 225.
FITZPATRICK, R. J. (1951). Proc. R. Soc. Med. 44, 870.
FITZPATRICK, R. J. (1957). Vet. Rec. 69, 713.
FOLEY, R. C. & REECE, R. P. (1953). Bull. Mass. agric. Exp. Sta. No. 468.
FOLEY, R. C., REECE, R. P. & LEATHEM, J. H. (1954). J. Anim. Sci. 13, 131.
FRANCIS, J. (1941). Vet. J. 97, 243.
FRANK, A. H. & BRYNER, J. H. (1952). J. Amer. vet. med. Ass. 121, 97.
GUNTER, J. J., COLLINS, W. J., OWEN, J., SORENSEN, A. M., SCALES, J. W. & ALFORD, J. A. (1955). Amer. J. vet. Res. 16, 282.
HAMMOND, J. (1927). The physiology of reproduction in the cow. London: Cambridge University Press.
HANCOCK, J. L. & KELLY, W. R. (1948). Vet. Rec. 60, 669.
HANSEL, W. & ASDELL, S. A. (1952). J. Anim. Sci. 11, 346.
HATCH, R. D. (1941). Amer. J. vet. Res. 2, 411.
HAUPT, H. (1930). Berl. tierärztl. Wschr. 46, 696.
HAYS, R. L. & VanDEMARK, N. L. (1951). J. Dairy Sci. 34, 496.
HAYS, R. L. & VanDEMARK, N. L. (1953). Endocrinology 52, 634.
JORDAN, W. J. (1952). J. comp. Path. 62, 54.
KAMPELMACHER, E. H. (1952). Rep. IInd Int. Congr. Physiol. anim. Reprod., Copenhagen, 1952. 2, 188.
KINGMAN, H. E. (1944). Amer. J. vet. Res. 5, 223.
KLIMMER, M., HAUPT, H. & ROOTS, E. (1929). Zbl. Bkt. (Orig.) 110, 62 and 111, 207.

KNORPP, G. (1928). Diss., Munich.
KRUPSKI, A. (1917). Schweiz. Arch. Tierheilk. 59, 1.
KUNST, C. (1911). Diss., Berne.
MAKHINYA, E. M. (1957). Sborn. Rabot vsesoyuz. Inst. eksp. Vet. p. 136.
MARTÍNEZ, M. G. (1955). Rev. Patronato Biol. anim., Madrid 1, 301.
MJULJBERG, V. M. (1937). Sborn. Rabot Leningrad vet. Inst. p. 240.
MOORE, G. R. (1954). Vet. Med. 49, 63.
MURPHEY, H. S. (1924). J. Amer. vet. med. Ass. 65, 598.
PALMER, R. (1948). Proc. 1st Internat. Congr. Physiol. & Path. Anim. Reprod. & Artif. Insem.
PLEBANI, R. (1954). Atti Soc. ital. Sci. vet., Cortina d'Ampezzo, 1953. 7, 508.
ROARK, D. B. & HERMAN, H. A. (1950). Res. Bull. Mo. agr. Exp. Sta. No. 455.
ROWSON, L. E. (1951). J. Endocrinol. 7, 260.
ROWSON, L. E. (1955). Brit. vet. J. 111, 334.
SIMON, J. & McNUTT, S. H. (1957). Amer. J. vet. Res. 18, 241.
SKJERVEN, O. (1953). Nord. VetMed. 5, 359.
SKJERVEN, O. (1954). Beretn. VII. Nord. Vetmøte p. 236.
SKJERVEN, O. (1956). Acta. endocr., Copenhagen Suppl. No. 26.
SMITH, F. R. & SHERMAN, J. M. (1939). J. infect. Dis. 65, 301.
SOHNLE, N. (1901). Mh. prakt. Tierheilk. 12, 337.
STRIKWERDA, R. (1956). Tijdschr. Diergeneesk. 81, 705.
SÝKORA, F. (1932). Klin. Spisy Školy Zvěrolékařské, Brno 9, 1.
SZÉKY, A. & DÓZSA, L. (1955). Acta. vet. Acad. Sci. hung. 5, 99.
SZÉKY, A. & DÓZSA, L. (1956). Acta. vet. Acad. Sci. hung. 6, 259.
TAGLIAVINI, A. (1934). Clin. vet., Milano 57, 3.
TERPSTRA, J. I. (1956). Proc. IIIrd Int. Congr. Anim. Reprod. Plenary Papers. p. 34.
TRIMBERGER, G. W. (1941). J. Dairy Sci. 24, 819.
VanDEMARK, N. L. (1953). in "Mammalian Germ Cells. A Ciba Foundation Symposium", London: Churchill, p. 159.
VanDEMARK, N. L. & HAYS, R. L. (1951). J. Anim. Sci. 10, 1083.
VanDEMARK, N. L. & HAYS, R. L. (1952). Amer. J. Physiol. 170, 518.
VanDEMARK, N. L. & HAYS, R. L. (1955). Amer. J. Physiol. 183, 510.
VanDEMARK, N. L. & MOELLER, A. N. (1951). Amer. J. Physiol. 165, 674.
VEELKEN, T. (1955). Inaug. Diss., Hanover.
VOLLMERHAUS, B. (1957). Zbl. VetMed. 4, 18.
WAGNER, H. (1927). Dtsch. tierärztl. Wschr. 35, 655.
WALL, S. (1915). Proc. Xth Int. vet. Congr., London 1914. 2, 292.
WEBER, A. F., MORGAN, B. B. & McNUTT, S. H. (1948). Amer. J. Anat. 83, 309.
WEETH, H. J. & HERMAN, H. A. (1952). Res. Bull. Mo. agr. Exp. Sta. No. 501.
WEINSTEIN, L., GARDNER, W. U. & ALLEN, E. (1937). Proc. Soc. exp. Biol., N. Y. 37, 391.
WEITZ, B. (1947). J. comp. Path. 57, 191.
WRENN, T. R., MOSS, S. & SYKES, J. F. (1954). J. Dairy Sci. 37, 671.
ZIETSCHMANN, O. (1921). Arch. Gynäk. 115, 201.
ZUPP, B. A. (1926). Vet. Pract. Bull., Iowa 8, 123. (quoted by Weber et al., 1948).

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Amoebic Infections in Animals

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INTRODUCTION

While the parasitic amoebae of man have been studied extensively, and the medical importance of human amoebiasis caused by Entamoeba histolytica is firmly established, our knowledge of the amoebae parasitic in domestic and wild animals of economic and public health importance is still fragmentary, and information about them is widely scattered in the world literature. This neglect is due mainly to two facts: first, most of the spontaneous amoebic infections of animals appear to be harmless to their hosts, and secondly, cases of disease caused by pathogenic amoebae are not commonly encountered.

It might be argued, therefore, that the study of these parasites is of no practical interest. However, there are several aspects of animal amoebiasis which merit serious consideration on the part of veterinary practitioners and laboratory workers. The most important of these is the public health significance of the disease in those animals (e.g. monkeys, dogs, rats) which are highly susceptible to infection with E. histolytica. Apart from their use in experimental studies of human amoebiasis, these animals sometimes harbour E. histolytica under natural conditions, and it is conceivable that they may serve as reservoirs or vehicles of human infection. Moreover, in the case of valuable animals (such as dogs), the control of spontaneous infections with E. histolytica becomes a problem of purely veterinary importance. Furthermore, the possibility of other species of amoebae causing clinical amoebiasis in animals should also be borne in mind. Finally, there remain the harmless commensal amoebae, the identification of which is essential for the differential diagnosis of the pathogenic forms. All these aspects of amoebic infections have been taken into consideration in the present review, in which the essential data regarding the amoebae recorded from various domestic and laboratory animals are brought together for the first time.

The parasitic amoebae of animals are represented by the same genera — and some species — as those parasitizing man, viz. Entamoeba, Endolimax and Iodamoeba, which are differentiated chiefly by the structure of the nucleus and cysts. They all inhabit the alimentary tract, the majority being found in the large intestine, while a few occur in the mouth or in the stomach. A host-list of the amoebae recorded from naturally infected animals of economic and public health importance, as well as from laboratory rodents, is given in the accompanying table. In this list,

the names of those amoebae which have been identified with other species are given in brackets, as their synonyms. However, as our knowledge of these parasites increases, the validity of some of the accepted species might have to be reconsidered.

For permission to use the figures illustrating this review, the writer is indebted to the authors named in the legends and to the publishers.

HOST-LIST OF AMOEBAE

HOSTS	HABITAT	AMOEBAE	SERIAL NO.
MACAQUE MONKEYS	Large intestine	Entamoeba histolytica (= E. nuttalli, E.duboscqi, E. cynomolgi)	5
	" "	Entamoeba coli (= E.legeri)	1
	" "	Entamoeba chattoni	8
	" "	Endolimax nana (= E. cynomolgi)	20
	" "	Iodamoeba bütschlii (= I. kueneni)	24
DOG	Large intestine	Entamoeba histolytica (= E. venaticum)	5
	Intestine	Entamoeba caudata	15
	"	Entamoeba coli	1
	Mouth	Entamoeba canibuccalis	18
	Intestine	Endolimax nana	20
CAT	Intestine	Entamoeba histolytica	5
HORSE	Intestine	Entamoeba equi	6
	Large intestine	Entamoeba gedoelsti (= E. intestinalis)	17
	Mouth	Entamoeba equibuccalis	19
DONKEY	Mouth	Entamoeba equibuccalis	19

HOSTS	HABITAT	AMOEBAE	SERIAL NO.
CATTLE	Stomach	Entamoeba bovis	9
	Intestine	Entamoeba histolytica	5
BUFFALO	Intestine	Entamoeba bubalus	10
GOAT	Intestine (?)	Entamoeba wenyoni	2
	Large intestine	Entamoeba debliecki	12
	Stomach, intestine	Entamoeba caprae	16
	Intestine	Entamoeba dilimani	13
SHEEP	Intestine	Entamoeba ovis	14
	Stomach	Entamoeba caprae	16
PIG	Large intestine	Entamoeba histolytica	5
	" "	Entamoeba coli	1
	" "	Entamoeba suis (= E. polecki)	11
	" "	Entamoeba debliecki (= E. polecki)	12
	" "	Endolimax nana	20
	" "	Iodamoeba bütschlii (= I. suis)	24
RAT	Caecum	Entamoeba histolytica	5
	"	Entamoeba muris	3
	Colon	Endolimax ratti	21
MOUSE	Caecum	Entamoeba muris	3
RABBIT	Caecum	Entamoeba muris (= E. cuniculi)	3
	"	Endolimax sp.	20

HOSTS	HABITAT	AMOEBAE	SERIAL NO.
GUINEA-PIG	Caecum	Entamoeba muris (= E. cobayae)	3
	Large intestine	Endolimax caviae	22
FOWL, TURKEY	Caecum	Entamoeba gallinarum (= E. lagopodis)	4
	"	Endolimax gregariniformis (= E. janisae)	23
GUINEA-FOWL	Caecum	Endolimax gregariniformis (=E. numidae)	23
DUCK	Intestine	Entamoeba anatis	7

DESCRIPTION

I. Genus Entamoeba Casagrandi & Barbagallo, 1895.

In amoebae of this genus the nucleus has a relatively small karyosome and chromatin granules lining the nuclear membrane (peripheral chromatin).

In the description which follows the species of Entamoeba are arranged in groups, according to the number of nuclei in the mature cyst (8, 4, 1), followed by species in which cysts have not been recorded.

(a) ENTAMOEBAE WITH 8-NUCLEATE CYSTS.

1. Entamoeba coli (Grassi, 1879).

Hosts: man, pig, monkeys, dog; cosmopolitan. Description: nucleus with eccentric karyosome and fairly coarse peripheral chromatin granules; size of trophozoites 20–30 μ; cysts, 10–30 μ in diameter, containing chromatoid bodies and glycogen, which is abundant in early stages but absent in mature octonucleate cysts (Fig. 3 a,b). Host-Parasite Relations: Non-pathogenic parasite living in the large bowel. (Dobell, 1919; Hoare, 1949.)

2. Entamoeba wenyoni Galli-Valerio, 1935.

Octonucleate cysts of this amoeba, measuring 6–9 μ in diameter, were recorded from the faeces of goats in England and Switzerland. (Wenyon, 1926; Galli-Valerio, 1935.)

3. Entamoeba muris (Grassi, 1879).

Hosts: Common parasite of rats and mice, also described under different names from other rodents (e.g. E. cuniculi Brug, 1918 in rabbits and hares, E. cobayae Walker, 1908 in guinea-pigs, etc.); cosmopolitan. Description: Resembles E. coli in structure of nucleus and cysts; size of trophozoites 12–30 μ; cysts, 9–23 μ in diameter (Fig. 3 c,d). Host-Parasite

Relations: inhabits caecum, non-pathogenic. (Neal, 1950.)

4. Entamoeba gallinarum Tyzzer, 1920.

Hosts: fowl and turkey; U.S.A. Description: nucleus with eccentric karyosome; size of trophozoites 18–20 μ x 12–14 μ, cysts, 15.5–17.5 μ in diameter (Fig. 3 e,f). Host-Parasite Relations: found in caecal contents and on surface of mucosa, non-pathogenic. (McDowell, 1953.)

(b) ENTAMOEBAE WITH 4-NUCLEATE CYSTS.

5. Entamoeba histolytica Schaudinn, 1903.

Hosts: man, monkeys, pig, dog, rats. Description: this amoeba is represented by two races (probably distinct species) differing in size and pathogenicity; nucleus in both has a small central karyosome and finely granular peripheral chromatin; size of trophozoites 18–40 μ (large race), 5–11.5 μ (small race); diameter of cysts, containing chromatoid bodies and glycogen, 7–16 μ (large race), 4–9.5 μ (small race). (Fig. 3 g, h,i,k.) Host-Parasite Relations: the large race (E. histolytica proper) is a pathogenic parasite inhabiting the large bowel. Infection in man may be symptomless (in carriers) or it produces a diseased condition, with ulceration of the gut wall and clinical symptoms varying from minor intestinal disorder to acute amoebic dysentery. There may also be metastatic invasion of the liver and other organs, giving rise to abscesses. The small race (E. hartmanni) is not pathogenic. (Hoare, 1949, 1952, 1958; Anderson et al., 1953.)

A number of animals (e.g. cats, dogs, rats, guinea-pigs and rabbits) are susceptible to experimental infection with human strains of E. histolytica. Under natural conditions, this parasite occurs in macaque monkeys and pigs, in which it produces symptomless infections, and in dogs and rats, to which it may be pathogenic.

Amoebiasis in Monkeys. It is well known that various primates naturally harbour amoebae indistinguishable from the human parasites (cf. Kessel, 1928a), but only spontaneous infections with E. histolytica occurring in macaque monkeys (Macaca spp.) are of some public health importance, owing to the fact that enormous numbers of these animals are now imported to different countries for use in the preparation of poliomyelitis vaccine, and also because in countries of their origin they might act as reservoir hosts of this amoeba. In both cases it is conceivable that human beings handling these monkeys, or otherwise exposed to contamination with their faeces, might contract amoebiasis, although in the macaques themselves the infection is typically symptomless. In this connexion it should be noted that the identity of E. histolytica in simian and human strains has been demonstrated by cross-infections (Dobell, 1931; Knowles & Das Gupta, 1934), which proved that the amoebae of macaques and man are interchangeable between these hosts.

When examining monkeys for intestinal amoebae, it is essential that E. coli [synonym E. legeri (Mathis, 1913): see No. 1], E. chattoni (see No. 8), Endolimax nana (synonym E. cynomolgi Brug, 1923: see No. 20) and Iodamoeba bütschlii [synonym I. kueneni (Brug, 1921): see No. 24], with which macaques may be naturally infected, should be differentiated from E. histolytica [synonyms: large race = E. nuttalli Castellani, 1908; small

race = E. duboscqi (Mathis, 1913), E. cynomolgi Brug, 1923].

Amoebiasis in Dogs. Spontaneous infection with E. histolytica has been recorded in dogs from North Africa, India, Indo-China, Indonesia, China, the U.S.A. and elsewhere. The amoebae are found throughout the large bowel, in which they may invade the wall, producing extensive erosion of the mucosa or deep ulcers extending into the muscularis (Fig. 1). The clinical course is variable: in some cases the infection is mild, terminating in spontaneous recovery; in others there is colitis, which may become chronic — lasting up to one year — or lead to a convalescent carrier state; lastly, some cases develop acute amoebic dysentery, with a fatal termination (Faust, 1932; Faust & Kagy, 1934; Chary et al., 1954; Ganapathy & Alwar, 1957). As in human amoebiasis, the disease may be complicated by the development of amoebic abscesses of the liver (Brumpt, 1949). Sometimes, e.g. in hunt packs in India (Ware, 1916; Boyd, 1931) and among army dogs in Indo-China (Chary et al., 1954), amoebic dysentery in dogs may assume epizootic proportions. In addition to overt amoebiasis, dogs may harbour E. histolytica without manifesting any signs of disease, as shown by Eyles et al. (1954), who detected cryptic infections in 8.4% of the dogs examined in Tennessee by the cultural method. It is therefore probable that canine amoebiasis is more widespread than is generally realized, especially in countries where canine ancylostomiasis is common and where there may be a tendency to attribute all cases of dysentery in dogs to infection with ancylostomes.

The susceptibility of dogs to experimental infection with human strains of E. histolytica, their close contact with human carriers in some reported cases, coupled with their coprophagic habits, seem to indicate that spontaneous canine amoebiasis is usually of human origin. Nevertheless, occasional transmission of canine infection to man cannot be excluded, especially in the case of chronic and symptomless infections, when cysts of E. histolytica are discharged by dogs (Andrews, 1932; Chary et al., 1954), while transmission from dog to dog has actually been observed (Chary et al., 1954). Canine amoebiasis is therefore of both public health and veterinary importance. (See table for other canine amoebae.)

Amoebiasis in Cattle. An unusual instance of bovine amoebiasis was reported from the Belgian Congo (Walkiers, 1930), in a locality (Isangi) where an epidemic of amoebiasis occurred among Africans. This outbreak was followed by an epizootic of dysentery in local cattle, in some of which the examination of faeces revealed numerous E. histolytica. It was thought that cattle contract amoebiasis when grazing on pastures contaminated with infected human faeces. In Dakar E. histolytica was found in the lungs of zebu cattle suffering from streptothricosis and bronchopneumonia (Thiéry & Morel, 1956). (See table for other bovine amoebae.)

Amoebiasis in Rats. Rats are susceptible to experimental infection with human strains of E. histolytica, and are widely used for testing amoebicidal drugs. Natural infections of rats with this amoeba have been reported from England, Russia, Iraq, Indonesia and the U.S.A. In infected rodents the amoebae are found in the caecum, where they either live as harmless commensals or invade the wall, producing superficial ulceration,

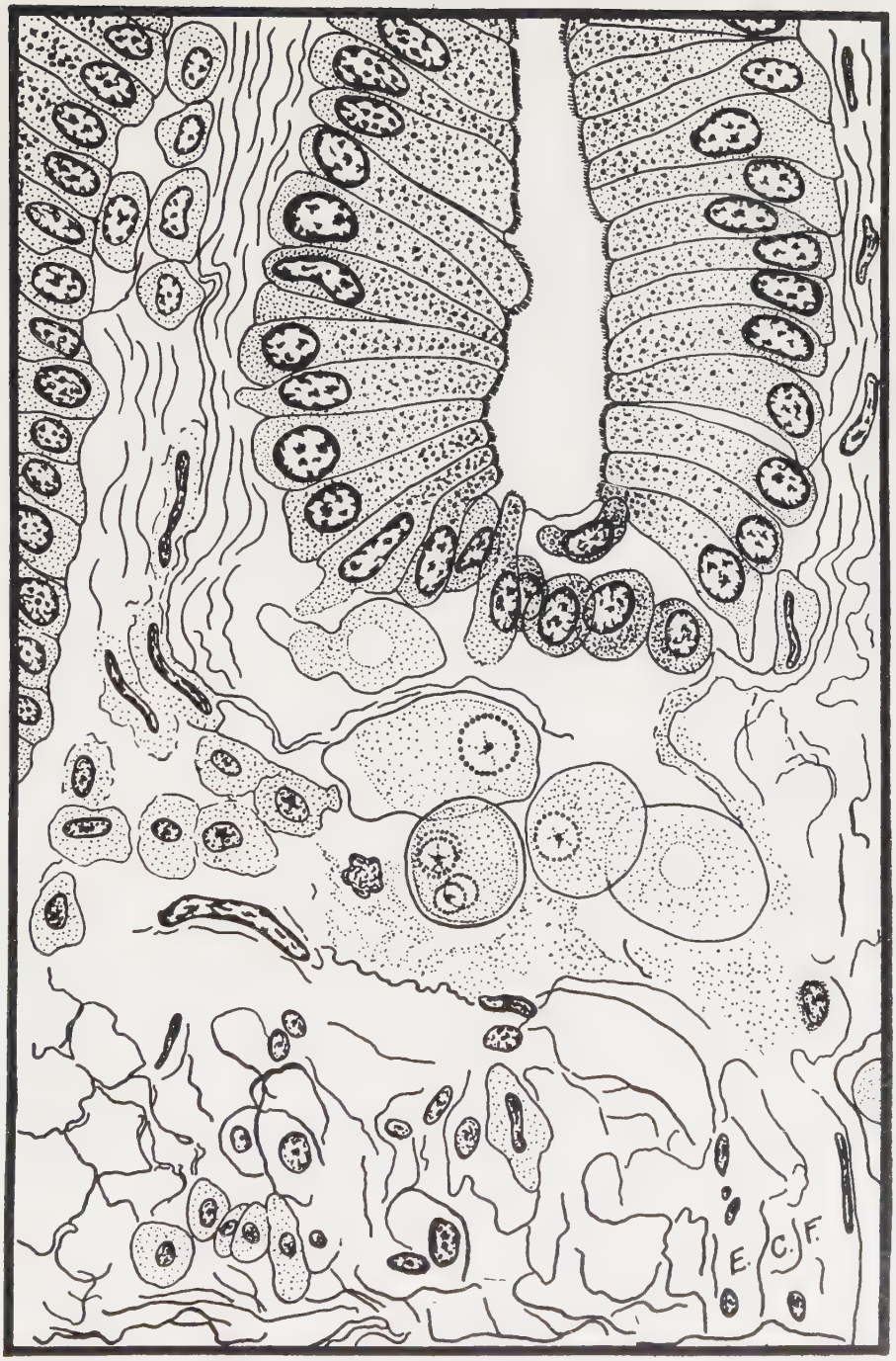

Fig. 1

Section of rectum from dog infected with Entamoeba histolytica, showing invasion of muscularis mucosae by amoebae. (x 1120)
(From Faust & Kagy, 1934.)

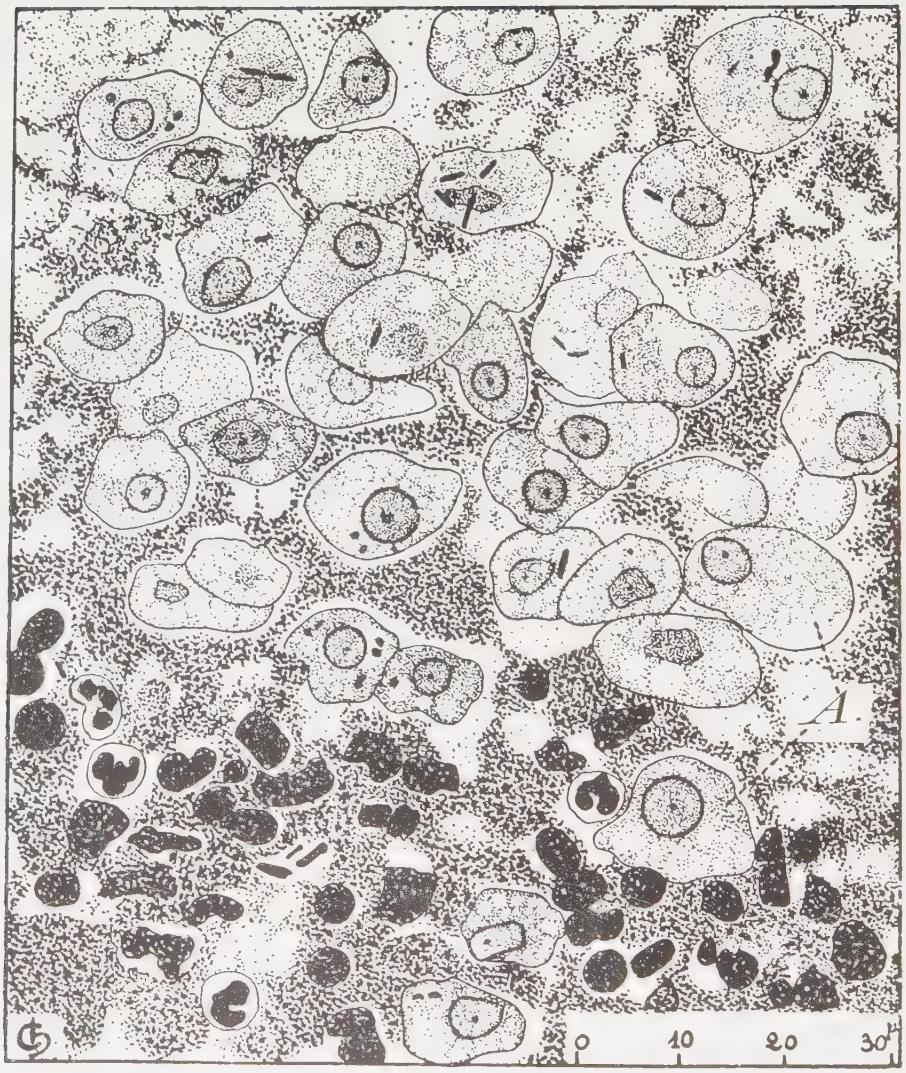

Fig. 2

Section of intestinal wall of pig, invaded by Entamoeba suis (A), in a case of swine fever. (From Brumpt, 1949.)

without dysenteric symptoms. Since in some instances spontaneously infected rats were associated with human carriers of E. histolytica, it is conceivable that they acquire the infection from such cases, and may also be of some epidemiological importance as vehicles in the transmission of amoebiasis to man. (Neal, 1948, 1951.) (See table for other amoebae of rats.)

6. Entamoeba equi Fantham, 1921.

Reported from the faeces of horses with signs of intestinal malaise; South Africa. Size of trophozoites 28 x 35 μ; cysts, 15–24 μ.

7. Entamoeba anatis Fantham, 1924.

Found in the caecum of domestic ducks; South Africa and the U.S.A. Nucleus with eccentric karyosome; size of trophozoites 14–22.5 μ; cysts, 13 x 14 μ (Fig.3,1). (Hegner, 1929.)

(c) ENTAMOEBAE WITH 1-NUCLEATE CYSTS.

8. Entamoeba chattoni Swellengrebel, 1914.

Non-pathogenic amoeba from the intestine of Old World monkeys, especially macaques (Macaca spp.). Nucleus with central karyosome; size of trophozoites 13 x 12 μ, 12 x 9 μ; cysts, 8–14 μ (mean ca. 11 μ) and 10–17 μ (mean 13 μ), representing two races (Fig. 3 m,n). (Salis, 1941.)

9. Entamoeba bovis (Liebetanz, 1905).

Non-pathogenic amoeba from the alimentary canal of cattle; Europe, North America, Korea, Japan. Nucleus with central karyosome; size of trophozoites 5–10 μ; cysts, 5–12 μ (Fig. 4 n,o). (Noble, 1950; Noble & Noble, 1952.)

10. Entamoeba bubalus Noble, 1955.

Non-pathogenic amoeba from alimentary canal of water buffalo or carabao (Bubalus bubalis); Philippine Islands. Nucleus with large irregular karyosome and uniform peripheral chromatin; average size of trophozoites 12 μ; cysts, 5–9 μ (average 8 μ). (Fig. 4 g,h.)

11. Entamoeba suis Hartmann, 1913.

(Synonym: E. polecki Prowazek, 1912, pro parte.)

Host: common parasite of pigs throughout the world. Description: nucleus with small central karyosome; size of trophozoites 12–25 μ; cysts, 12–15 μ (Fig. 4 a,b). Host-Parasite Relations: inhabits caecum; invasion of the gut wall by these amoebae (Fig. 2) was described by Theobald Smith (1910) and Brumpt (1949), but since in both cases the hosts were also suffering from swine fever, it is not clear whether this amoeba is normally pathogenic or whether its dormant virulence is activated by the concomitant virus infection; in any case, the effect of E. suis upon its host is in need of further study. (Hoare, 1940.)

12. Entamoeba debliecki Nieschulz, 1923.

(Synonym: E. polecki Prowazek, 1912, pro parte.)

Hosts: this amoeba is commonly found in pigs of most countries, and has also been recorded from goats in Holland, England and the U.S.A. Description: nucleus with large karyosome and irregular coarse peripheral chromatin; size of trophozoites 5–9 μ; cysts, 5–13 μ (Fig. 4 c–f). Host-Parasite Relations: found in intestine; probably non-pathogenic.

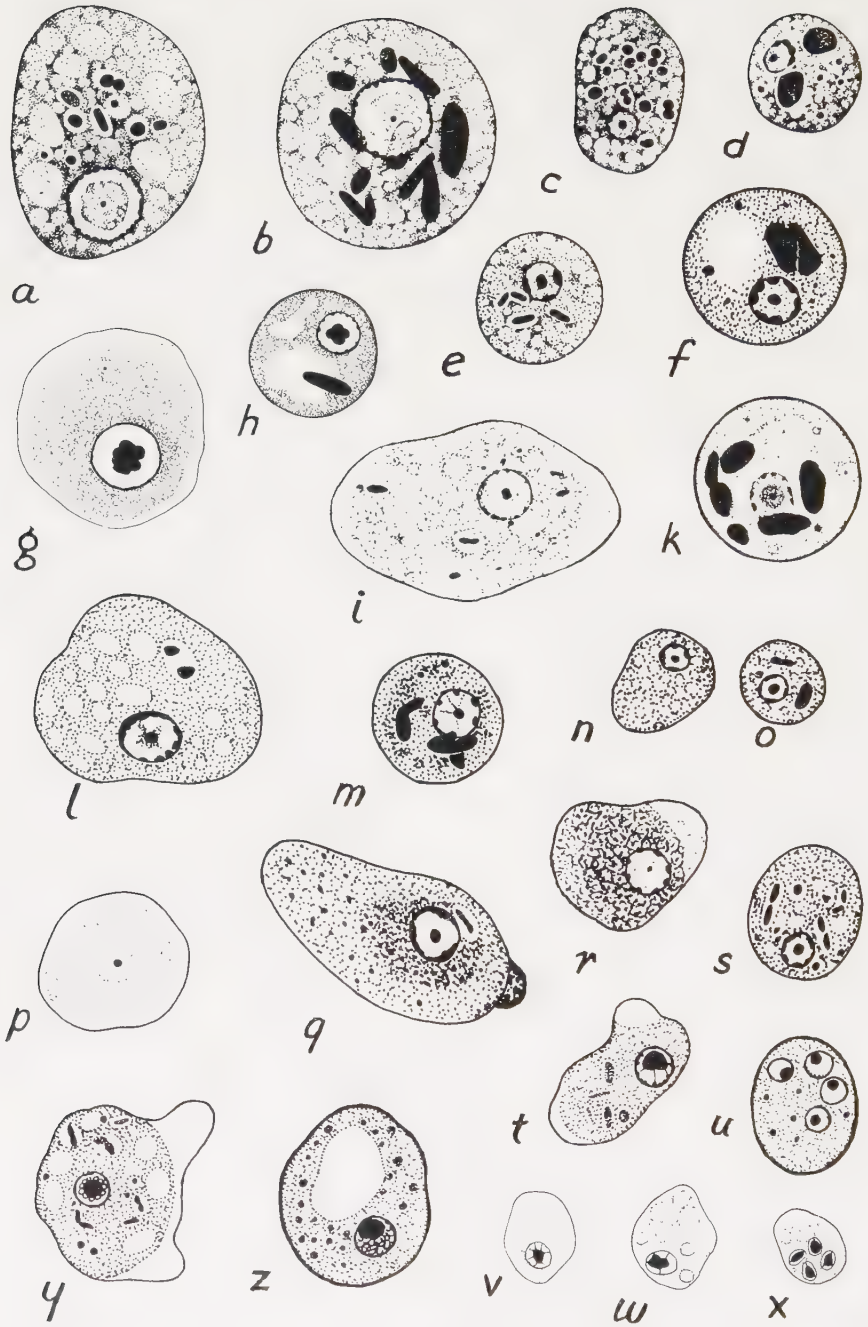

Fig. 4

Africa. Nucleus with eccentric karyosome; size of trophozoites, 34 x 25 μ; cysts unknown.

17. Entamoeba gedoelsti Hsiung, 1930 [Synonym: E. intestinalis (Gedoelst, 1911)].

Recorded from the large intestine of horses; Europe, South Africa, North America. Nucleus said to be of E. coli-type; size of trophozoites 6.5–12.5 x 6–11 μ (Fig 4 p); cysts unknown.

18. Entamoeba canibuccalis Simitch, 1938.

Reported from dogs in England and Yugoslavia. Similar to human oral parasite, E. gingivalis: nucleus with central karyosome; size of trophozoites 6–14 μ (Fig. 4 r); cysts unknown. Host-Parasite Relations: found in mouth in normal and pyorrhoeic condition, probably non-pathogenic. (Simitch, 1938a.)

19. Entamoeba equibuccalis Simitch, 1938 (Synonym: E. gingivalis var. equi Nieschulz, 1924).

Reported from the mouth (teeth and gums) of horses and donkeys; Holland and Yugoslavia. Resembles human oral parasite, E. gingivalis: size of trophozoites 8–14 μ (Fig. 4 s); cysts unknown. (Nieschulz, 1924; Simitch, 1938b.)

Legend to Fig. 4 (facing)

Amoebae in animals (x ca. 2000)
(Abbreviations: tr = trophozoite, cy = cyst)

a, b. Entamoeba suis: tr, cy (From Wenyon, 1926);
c–f. E. debliecki (c, d: tr, cy, in pig, from Wenyon, 1926; e, f: tr, cy, in goat, after Nieschulz, 1923, and Hoare, 1940);
g, h. E. bubalus: tr, cy (From Noble, 1955);
i, k. E. dilimani: tr, cy (From Noble, 1954);
l, m. E. ovis: tr, cy (After Nöller, 1922, and Triffitt, 1926);
n, o. E. bovis: tr, cy (After Nieschulz, 1922);
p. E. gedoelsti: tr (After Hsiung, 1930);
q. E. caudata: tr (After Carini & Reichenow, 1949);
r. E. canibuccalis: tr (After Simitch, 1938);
s. E. equibuccalis: tr (After Nieschulz, 1924);
t, u. Endolimax nana (= E. ratti): tr, cy (From Hoare, 1949);
v. E. caviae: tr (After Hegner, 1926);
w, x. E. gregariniformis: tr, cy (After Hegner, 1926);
y, z. Iodamoeba bütschlii: tr, cy (From Hoare, 1949).

II. Genus Endolimax Kuenen & Swellengrebel, 1917.

Amoebae of this genus are all non-pathogenic; they are small, their nucleus has a large irregular eccentric karyosome and no peripheral chromatin; mature cysts usually oval, 4-nucleate.

20. Endolimax nana (Wenyon & O'Connor, 1917).

Hosts: common in large intestine of man, macaque monkeys, pigs; also reported from dogs. Description: size of trophozoites 6–12 μ (average 8 μ); cysts oval, 6–9 x 5–7 μ (Fig. 4 t,u). (Hoare, 1949; Kessel, 1928a, b; Eyles et al., 1954.)

21. Endolimax ratti Chiang, 1925.

Reported from colon of laboratory rats in the U.S.A. Indistinguishable from human E. nana (Fig. 4 t, u): size of trophozoites 6–12 μ; cysts oval, 6–9 x 5–7 μ.

22. Endolimax caviae Hegner, 1926.

Reported from caecum and colon of guinea-pigs; U.S.A. Size of trophozoites 4.5–10 μ (Fig. 4 v); cysts unknown. (Nie, 1950.)

23. Endolimax gregariniformis (Tyzzer, 1920). (Synonyms: Pygolimax gregariniformis Tyzzer, 1920; Endolimax janisae Hegner, 1926).

Found in caecum of domestic fowl, turkey and guinea-fowl; U.S.A. Size of trophozoites 9.4 x 7.7 μ; cysts oval, 9.7 x 6.6 μ (Fig. 4 w, x). (McDowell, 1953.)

III. Genus Iodamoeba Dobell, 1919.

Amoebae of this genus are non-pathogenic; their nucleus has a large spherical karyosome surrounded by achromatic granules; mature cysts of irregular shape, uninucleate, with abundant glycogen.

24. Iodamoeba bütschlii (Prowazek, 1912).

Hosts: found in large intestine of man, pigs and macaque monkeys. Description: the human and animal parasites are indistinguishable, with general characters of the genus; size of trophozoites 5–20 μ; cysts, 9–15 μ (Fig. 4 y, z).

Relationship of human and porcine strains.

Iodamoeba bütschlii is a common parasite of pigs throughout the world, with an incidence of infection reaching 63% (in Brazil). The incidence among human beings is much lower: usually 0.3–5% but in some countries, e.g. Brazil, reaching 17% (Coutinho & Rabelo, 1956). Many authorities regard the pig as the reservoir host of human infection (Cauchemez, 1921; Wenyon, 1926; Coutinho & Rabelo, 1956) but there is no conclusive evidence of this connexion. On the contrary, working with Argentine strains, Bacigalupo (1938) found that piglets – though susceptible to infection with the porcine strain – were refractory to the human strain; and he detected no cases of infection among pig-handlers. From these observations the author concluded that man and pig harbour distinct races of Iodamoeba, which are not interchangeable between these hosts.

CONCLUSIONS

From the foregoing account it is evident that our present knowledge of amoebic infections in animals is incomplete. However, there are definite indications that clinical amoebiasis caused by Entamoeba

histolytica -- both acquired from human sources and spontaneous -- does occur in certain mammals, and it is conceivable that they might serve as reservoirs of human infection. Furthermore, the possibility of other species of amoebae being pathogenic cannot be excluded. It is hoped, therefore, that this review will serve a useful purpose, by stimulating interest in the amoebae of animals in general, and by promoting investigations on the epidemiology and host–parasite relations of infections with the pathogenic amoebae.

REFERENCES

ANDERSON, H.H., BOSTICK, W. L. & JOHNSTONE, H.G. (1953). Amebiasis: pathology, diagnosis and chemotherapy. Springfield, Ill.: Charles C. Thomas.

ANDREWS, J. (1932). Amer. J. trop. Med. 12, 401.

BACIGALUPO, J. (1938). Rev. Med. trop., Habana 4, 141. [Abst. in Trop. Dis. Bull. 36, 600 (1939).]

BOYD, J. S. K. (1931). J. R. Army Med. Cps 56, 1.

BRUMPT, E. (1949). Précis de parasitologie. 6th Edit., Vol. 1. Paris: Masson et cie.

CARINI, A. & REICHENOW, E. (1949). Z. Tropenmed. u. Parasit. 1, 102.

CAUCHEMEZ, L. (1921). Bull. Soc. Path. exot. 14, 321.

CHARY, R., GONZALÈS, P., LAPOUGE & PÉRON. (1954). Rev. Cps vét. Armée 1, 26. [Abst. in Vet. Bull., Weybridge 25, 2751 (1955).]

CHIANG, S. F. (1925). Proc. Nat. Acad. Sci. 2, 239.

COUTINHO, J.O. & RABELO, E.X. (1956). Arq. Hig., S. Paulo 10, 71. [Abst. in Trop. Dis. Bull. 54, 1084 (1957).]

DOBELL, C. (1919). The amoebae living in man. London: Bale, Sons & Danielsson.

DOBELL, C. (1931). Parasitology 23, 1.

EYLES, D. E., JONES, F. E., JUMPER, J. R. & DRINNON, V. P. (1954). J. Parasit. 40, 163.

FANTHAM, H. B. (1921). S. Afr. J. Sci. 18, 164.

FANTHAM, H. B. (1923). S. Afr. J. Sci. 20, 493.

FAUST, E. C. (1932). Amer. J. trop. Med. 12, 37.

FAUST, E. C. & KAGY, E. S. (1934). Amer. J. trop. Med. 14, 221.

GALLI-VALERIO, B. (1935). Schweiz. Arch. Tierheilk. 77, 643.

GANAPATHY, M. S. & ALWAR, V. S. (1957). Indian vet. J. 34, 427.

HEGNER, R. (1929). Amer. J. Hyg. 10, 33.

HOARE, C. A. (1940). Parasitology 32, 226.

HOARE, C.A. (1949). Handbook of medical protozoology. London: Baillière, Tindall & Cox.

HOARE, C. A. (1952). Exp. Parasit. 1, 411.

HOARE, C. A. (1958). Rice Inst. Pamphl. 45 (1), 23.

HSIUNG, TA-SHIH. (1930). Iowa St. Coll. J. Sci. 4, 359.

KESSEL, J. F. (1928a). Univ. Calif. Publ. Zool. 31 (13), 275.

KESSEL, J. F. (1928b). Amer. J. trop. Med. 8, 481.

KNOWLES, R. & DAS GUPTA, B. M. (1934). Indian med. Gaz. 69, 390.

McDOWELL, S., Jr. (1953). J. Morph. 92, 337.

NEAL, R. A. (1948). J. Hyg., Camb. 46, 90.
NEAL, R. A. (1950). Parasitology 40, 343.
NEAL, R. A. (1951). Trans. R. Soc. trop. Med. Hyg. 45, 363.
NIE, D. (1950). J. Morph. 86, 381.
NIESCHULZ, O. (1924). Arch. wiss. prakt. Tierheilk. 51, 41.
NOBLE, E. R. (1950). Univ. Calif. Publ. Zool. 47(13), 341.
NOBLE, G. A. (1954). Philippine J. Sci. 83, 113.
NOBLE, G. A. (1955). J. Prot. 2, 19.
NOBLE, G. A. & NOBLE, E. R. (1952). J. Parasit. 38, 571.
SALIS, H. (1941). J. Parasit. 27, 327.
SIMITCH, T. (1938a). Ann. Parasitol. 16, 251.
SIMITCH, T. (1938b). Riv. Parassitol. 2, 183.
SMITH, T. (1910). J. med. Res. 23, 423.
SWELLENGREBEL, N. H. (1914). Geneesk. Tijdschr. Nederl.-Indië 54, 420.
THIÉRY, G. & MOREL, P. (1956). Rev. Élev. 9, 343. [Abst. in Vet. Bull., Weybridge 27, 2967 (1957).]
WALKIERS, J. (1930). Ann. Soc. belge Méd. trop. 10, 379.
WARE, F. (1916). J. comp. Path. 29, 126.
WENYON, C. M. (1926). Protozoology, Vol. 1. London: Baillière, Tindall & Cox.

The Significance of Carbohydrate in Ruminant Metabolism

BY

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In recent years the metabolism of glucose has been stated to be quantitatively less important in ruminants than in other mammals (Baxter et al., 1955). This review will examine the reasons for this belief, how far it is true, and some of the implications which follow.

In ruminants some of the functions of carbohydrate are taken over by fatty acids. The metabolism of fatty acids will therefore be examined in so far as this bears on the subject.

Origin of the carbohydrate. In the non-ruminant there are two sources — the diet and gluconeogenesis. The relative importance of the two can vary greatly. It may be appropriate to point out that, although convenient, it is often misleading to refer to "non-ruminants" as if they were a homogeneous group. For instance the wild carnivores probably ingest only trivial quantities of carbohydrate but domestic dogs and cats have a substantial carbohydrate intake. Man is often a greater eater of carbohydrate than many animals. But the rabbit and the horse have several features in common with ruminants; for them the caecum may play a part analogous in certain respects to the rumen; of critical importance for ruminants, however, is the site of fermentation in the gastro-intestinal tract. The distinctive feature is that after a test meal of glucose, hyperglycaemia develops in the non-ruminant but is not readily demonstrable in the ruminant (e.g. Holmes, 1952). This is because the micro-organisms of the rumen ferment carbohydrate before it reaches the small intestine.

Carbohydrate digestion and absorption. In non-ruminants there may be some salivary digestion in the mouth but most occurs in the stomach. Disaccharides (e.g. maltose), oligo-saccharide and polysaccharides such as starch and glycogen are all broken down to glucose, fructose etc. by acidic hydrolysis. Cellulose and lignin are not digested in the stomach; there is some evidence however of cellulose degradation in the caecum or colon in the rat (Conrad, Watts, Iacono, Kroybill & Friedemann, 1958), and the same must hold for the herbivores.

While glucose can be absorbed from the stomach, the main absorption site is the small intestine. Recently it was suggested much carbohydrate might be absorbed as some unknown small molecule (Hestrin-Lerner & Shapiro, 1954) but this appears to have been disproved by Kiyasu et al. (1956) and it seems that glucose is mainly absorbed as such.

In ruminants carbohydrate is fermented rather than digested in the rumen. This fermentation, leading to production of volatile fatty acids, is generally recognized, since the pioneer work of Sir Joseph Barcroft and his colleagues (Barcroft, McAnally & Phillipson, 1944).

Because of this fermentation ruminants can digest a much wider range of complex carbohydrates. There is extensive degradation of cellulose, pentosans and pectic substances, all of which may form a significant part of the food of ruminants.

Simple sugars are attacked, often rapidly, by both rumen protozoa and bacteria. The fermentation of glucose has been studied extensively (e.g. Elsden, 1945; Heald & Oxford, 1953). The products were identified as volatile fatty acids – particularly acetic and propionic (as well as rumen gases such as carbon dioxide and hydrogen) but significant amounts may first be stored, as carbohydrate within the micro-organisms (Oxford, 1951). Other simple sugars, such as maltose, lactose and galactose, are fermented more slowly (Phillipson & McAnally, 1942). The speed of fermentation depends to some extent on the diet. Elsden (1945) showed that rumen micro-organisms of the sheep fermented glucose much more rapidly when the quality of the dietary hay was improved.

Starch is also rapidly attacked and here too the main ultimate products are the volatile fatty acids. Lactic acid appears also, particularly when there is a sudden change from a low starch to high starch ration, and may lead to severe digestive disturbances (Scarisbrick, 1954). Under most feeding conditions lactic acid is only a transitory intermediate. Starch, as well as providing volatile fatty acids for the host animal, is important in the development of the rumen flora; its wasteful degradation (as compared to its utilization by non-ruminants) is the price paid for the ability to digest cellulose.

Cellulose. Its degradation by micro-organisms has been studied widely (see Oxford, 1955). The relative contribution of protozoa and bacteria is uncertain. Hungate (1943) showed the ability of certain rumen protozoa to attack cellulose, but Van der Wath & Myburgh (1941) found that sheep digested cellulose readily in the absence of protozoa.

The details of the degradation of cellulose are not clear. In vitro degradation is slow when a pure culture of cellulolytic bacteria is used. It seems likely that a number of species of bacteria are required for effective cellulolysis. Halliwell (1957) made some important advances using mixed rumen micro-organisms and investigated the initial stages of cellulose degradation.

Cellulose digestion is much stimulated by the presence of certain higher volatile fatty acids – both straight and branched chain (Bentley et al., 1954).

Other polysaccharides.

Pentosans such as xylan, which may form 16–20% of the dry weight of grass, are also digested extensively (Marshall, 1949). Howard (1955) showed that wheat flour pentosans are readily attacked by mixed rumen bacteria. Lewis (personal communication) showed that grass fructosan is readily fermented in the rumen. In each case, the main products of

fermentation are the volatile fatty acids.

Pectin and Pectic acid, which occur in good hay, have been shown to be more effectively digested than cellulose by sheep (Michaux, 1951). Oxford, (1956) emphasized the ability of rumen micro-organisms to attack non-cellulose carbohydrates such as fucoidin, laminarin etc. which constitute much of the dry weight of seaweed. Such material could be of poor nutritive value to non-ruminant animals. Pigden & Stone (1952) showed that although lignin is generally believed not to be attacked in the rumen, there is some digestion of the lignin from monocotyledonous crops. For these latter cases (pectin, fucoidin, lignin etc.) the products of degradation have not been identified.

Digestive products. It will be seen that although the digestion of different carbohydrates in the rumen follows different pathways, the ultimate products of a wide range of substrates are the volatile fatty acids -- particularly acetic, propionic and butyric, and these provide much of the energy supply of ruminants.

While the amounts in the rumen may be very high, little occurs in the abomasum (Phillipson & McAnally, 1942) and it is now certain that this is due to considerable absorption into the blood stream direct from the rumen.

Higher volatile acids -- both straight and branched chain -- are also found in the rumen, e.g. 2-methyl-butyric and iso-valeric and iso-butyric (Annison, 1954a). It is probable that the branched-chain acids are produced mainly by the deamination of amino-acids during the degradation of protein (El-Shazly, 1952; Annison, 1954a). They probably supply only a fraction of the energy required by the ruminant, since Annison et al. (1957) were unable to detect any acids higher than butyric in the portal blood of sheep even when these were fed casein. Studies by McCarthy et al. (1958a) with the isolated perfused goat rumen suggested that about 2.g. enter the circulation daily.

The acids may be of considerable importance in the rumen, since Bentley et al. (1954) showed that in vitro they stimulate cellulose digestion. Formic acid is found in negligible amount in the rumen. For this reason, Annison (1954b) considers that the formate in ruminant blood is endogenous. The findings of McCarthy, Shaw & Lakshmanan (1958b) suggest that it arises in the liver and is utilized by the rumen epithelium.

Acetic, Propionic and Butyric acids. A number of attempts have been made to estimate the amounts of these acids made available by fermentation. Barcroft and his colleagues (see Barcroft, 1945) gave the first estimates. Using anaesthetized sheep, they determined the difference in total volatile acid content between the blood of the carotid artery and the ruminal vein. The average difference multiplied by their estimate of blood flow suggested about 100 g. volatile fatty acids were produced in 24 hours, which would account for a substantial fraction of the total energy requirement of a sheep.

This technique was improved by Schambye (1951). Using the London cannula technique he took samples of portal blood in conscious sheep. He later (1955) estimated the portal flow by the dye dilution technique. Estimates of total volatile fatty acid production were 1–2 mM/min., or

about 150 g./day.

Annison et al. (1957), by inserting a polythene catheter in the portal vein of sheep, were able to take large samples of portal blood (15–20 ml.), to partition the blood volatile fatty acids (VFA), and to estimate absorption of acetic, propionic and butyric acid independently.

Acetate production was estimated at about 100 g./day, propionate 20–30 g; butyrate was not found in portal blood. This can be explained by the observations of Pennington (1952), who found that in vitro the rumen epithelium converts butyric acid to beta-hydroxy butyric acid. In agreement with this Annison et al. found concentrations of ketone bodies in portal blood 1–2 mg./100 ml. greater than in carotid blood. Assuming that this accounts for all the butyrate absorbed from the rumen, their figures suggest that about 10 g. butyrate/day are absorbed. Fegler & Hill (1958) suggested that Schambye's average values of blood flow in the portal vein of sheep are too low, and when this is taken into account the estimate of VFA absorbed from the gastro-intestinal tract is increased still further.

Conrad (H. R.) et al. (1958) used a similar technique for studies of VFA absorption in the anaesthetized calf. Acetate production over a 24-hour period averaged 300–600 g./day and propionate about 40% of this, for animals weighing about 100 kg.

A disadvantage of this technique is the difficulty of measuring portal flow at the same time as arterio-venous difference in VFA content. Since portal flow may vary considerably this is, theoretically, a serious drawback. It also has the disadvantage of requiring a surgical preparation which is difficult to maintain for any length of time, and may be inconvenient to set up when estimates are required of VFA production under very variable conditions. Attempts have also been made to estimate VFA production rates by following changes in the relative proportions of the acids in the rumen (Gray & Pilgrim, 1951); or by comparing changes in samples of rumen contents incubated in vitro with those occurring in the rumen (Carroll & Hungate, 1954; Halse & Velle, 1956). The system is so complex however, that it is doubtful if the estimates are reliable. Annison & Lewis (1959) discussed difficulties in interpreting the results.

But results obtained with this method by Balch (1958) and Stewart & Schultz (1958) are quoted, since apart from the estimates of Carroll & Hungate they are the only ones available in mature cattle. They estimated that up to 3 kg. total VFA may be produced daily. About half this was acetate and most of the remainder propionate and butyrate, production of butyrate being nearly as high as that of propionate in some circumstances. These values are only approximate, since Balch, for instance, found that one of his cows produced more acid than another (with the same food intake) and that ground hay yielded more propionate than long hay.

Recently, Annison & Lindsay (1958) described a promising technique for the estimation of acetate production. Acetate labelled with C^{14} is infused intravenously at a constant rate until the specific activity (units radioactivity/mM) of blood acetate reaches a constant value. In the steady state, acetate entering the blood (mM/min.) equals

$$\frac{\text{unit activity/min. infused}}{\text{units activity/mM found in blood}}$$

Acetate was estimated to enter the blood of sheep at about 1.5–2.0 mM/kg./hour. Lindsay & Ford (in preparation) found even higher rates in pregnant sheep feeding on winter grass and concentrates. It would be valuable to determine propionate and butyrate production by this technique.

To sum up, it appears that sheep, in which data may be more reliable, absorb daily 100–200 g. acetate, 30–40 g. propionate and 10–15 g. butyrate. Cattle absorb 1,500 g. acetate, 500–800 g. propionate and 300–600 g. butyrate. In view of the technique used in obtaining the figures for cattle, these should be treated with reserve.

Glucose. It is interesting to compare these figures with the amounts of glucose absorbed. It was thought likely at one time that carbohydrate stored in bacteria and protozoa might escape fermentation and be carried into the abomasum and small intestine, where acidic and enzymic digestion would liberate glucose, to be absorbed in the small intestine. This possibility was examined by Heald (1952). From estimates of the concentration of material carried into the abomasum which yielded glucose on acid hydrolysis, and the flow rate through the duodenum, he concluded that in sheep fed hay, no more than 20 g. glucose per day could be produced in this manner, and the figure might well be less.

McClymont & Scarisbrick (cited by McClymont, 1951) suggested an even smaller figure for sheep fed kale and hay -- 5 to 6 g. in 24 hours.

The studies of Annison et al. (1957) and Schambye (1951), cited above, also provide evidence on the amount of glucose conveyed from the gastro-intestinal tract. In both cases the portal/carotid difference in glucose concentration was small and probably not significantly different from zero. While it seems clear therefore that exogenous glucose cannot contribute substantially to the total energy requirement of ruminants, it would be a mistake to suppose that it has been established that no glucose is absorbed from the gastro-intestinal tract. With a portal/carotid difference of only 3 mg./100 ml., assuming a portal flow rate of about 1 litre/min., 45 g. glucose per day would be absorbed. As will be shown later, this is probably nearly half the daily glucose requirement of the sheep. On occasion Schambye (1951) recorded carotid/portal difference as high as 9 mg./100 ml.: thus with certain diets, and in young ruminants, (Barhydt & Dye, 1957) small quantities of carbohydrate may be absorbed from the small intestine. It may be of interest to note that Taylor (personal communication) finds that the pancreatic juice of the sheep contains an amylase of the same order of activity as that found, for instance, in the pancreatic juice of the dog.

Lactic acid. The only other small molecule likely to be provided in significant amounts by the diet is lactic acid. We have seen that lactic acid is produced normally when readily fermentable carbohydrate is attacked; nevertheless it is commonly regarded only as a transitory intermediate. It would be a mistake to suppose this is invariably the case. Hueter et al. (1956) have shown that sodium lactate added to the rumen leads to a large rise in blood lactic acid, and gave reasons for supposing this response was not due to a large influx of propionate. When sheep were turned on to fresh spring pasture, where an enormous amount of readily fermentable carbohydrate is available, high levels of rumen

lactic acid and raised levels of blood lactic acid were observed before the animals became adapted to the new diet (Annison et al., 1959). Thus in some circumstances at least, lactate may contribute to the energy requirements of ruminants. Generally however, lactate production is deleterious. Scarisbrick (1954) showed that excessive lactate production depressed rumen pH and causes severe digestive disorders.

In concluding this section on carbohydrate digestion in the rumen, it should be pointed out that there is considerable interaction between carbohydrate and protein digestion. It has already been mentioned that the branched-chain fatty acids produced in protein breakdown may assist the digestion of cellulose; in contrast, the active fermentation of starch is of value in protein metabolism. Lewis & McDonald (1958) showed that protein supplements were better utilized by sheep when readily fermentable carbohydrate was also given.

Glucose requirements of ruminants. The only satisfactory methods for the determination of the turnover rate of glucose in the intact animal use labelled glucose.

While many of such investigations have been reported in dogs and rats, the only one in ruminants yet published is that of Baxter et al. (1955), in the lactating cow. These workers made a single intravenous injection of labelled glucose, and followed the decline in the specific activity (radioactivity/unit weight) of plasma glucose. The rate at which this falls is a measure of the rate at which unlabelled glucose enters the circulation. Their estimate was about 150 mg./kg./hour. Expressed in terms of body area this is similar to values obtained in the non-lactating rat and dog. The authors drew attention to technical reasons why their estimate might be too high, and Steele et al. (1956) showed that in dogs the technique used by Baxter et al. overestimates glucose turnover. Thus, while at present no accurate figures can be given for glucose turnover, a value around 100–200 mg./kg./hour would seem reasonable, or about 100 g./day for a sheep and 1 kg./day for a cow. These figures are based on estimates for a lactating cow, where as will be shown later, the glucose requirement of lactation may form a considerable fraction of the total glucose requirement. It seems impossible to decide how far such a figure applies to other conditions.

Gluconeogenesis. If the sheep requires 100 g. of glucose and the cow 1 kg. daily, clearly the amount in the diet is inadequate. It appears that not more than 5–20 g. glucose/day is available to sheep from the gastro-intestinal tract and under most conditions this estimate is probably too high. Most of the glucose must come from gluconeogenesis.

Sources (1) Lactate. In laboratory animals, blood lactate is known to be very efficiently converted to glycogen by the liver, (Cori et al., 1930). Evidence discussed earlier however, suggests that while this may in special circumstances make some contribution to glucose requirement, in general lactic acid coming from the rumen is not an important source. Blood lactate of course is probably mainly derived from muscle glycolysis, hence its conversion to glycogen does not lead to a net synthesis by

the body as a whole.

(2) Volatile fatty acids. From studies with laboratory animals, it has generally been accepted that fatty acids of chain length with an odd number of carbon atoms, e.g. propionate (C_3), can be converted to carbohydrate, whereas acids with even numbers of carbon atoms (acetic (C_2), butyric (C_4)) are not used for synthesis of carbohydrate (Deuel, 1957). Studies with ruminants have on the whole supported this view. Five main techniques have been used:

(I) The acid was administered either via the rumen or intravenously and changes in blood sugar noted. Increases were assumed to indicate transformation of the fatty acid to glucose.

(a) Changes in blood sugar after a single intravenous dose of a fatty acid are unreliable evidence. Ash et al. (1958) showed that after administration of propionate and butyrate, (i) more sugar may be released than can be accounted for, assuming complete conversion to glucose (ii) when labelled fatty acids are administered, the glucose released is labelled to only a trivial extent. Unpublished data of these authors indicate that the effect is mainly due to sympathetic stimulation, since with butyrate at least, the effect is much reduced by splanchnicotomy or treatment with adrenergic blocking agents.

(b) More convincing results are obtained when a fatty acid is slowly dripped into the rumen and changes in blood sugar followed. Schultz & Smith (1951) and Clarke & Malan (1956) dripped solutions of fatty acids into the rumen of goats and sheep respectively. Consistent blood sugar rises were found only when the administered solutions contained propionate. Johnson (1955) using goats and Armstrong & Blaxter (1957) using sheep, gave intra-ruminal infusions lasting up to 48 hours. Sustained rises in blood sugar were found only when propionate was administered. An indirect stimulation of glucose output seems improbable under these conditions, and such experiments provide some of the best evidence that propionate is glucogenic in ruminants.

(II) Intravenous administration of the acid relieves insulin induced convulsions in the adrenalectomized or splanchnicotomized animal.

Propionate and butyrate, but not acetate, were effective in sheep, using this technique (Reid, 1951a; Potter, 1952). In both cases relief of symptoms is due to a large rise in blood sugar. This technique is clearly more reliable than the previous one, (Ia). As indirect stimulation of glycogen breakdown in the liver is possible, before results obtained by this technique can be considered unequivocal it should be established by the technique of Ash et al. that there is substantial conversion of labelled fatty acid to the glucose appearing in the blood.

(III) Sheep were treated with phlorhizin (which probably acts mainly by interfering with renal reabsorption of glucose) and starved until the glucose/nitrogen ratio in the urine was constant. The amount of extra glucose appearing in the urine after administration of the fatty acid provided an estimate of conversion to carbohydrate. Phillipson (1947), using this technique found that, after giving acetate plus propionate, extra glucose appeared corresponding to 40% conversion of acetate (he assumed 100% conversion for propionate). Acetate alone gave a small response. Jarrett & Potter (1950) obtained irregular results. Three of 15 treated

sheep gave evidence of 30–40% conversion of acetate to glucose. Unfortunately, as the workers were aware, this technique gives only equivocal results. It is impossible to be sure that the extra glucose is in fact coming from the administered acid unless very complicated balance experiments are carried out; and the animal is in such abnormal condition that the significance of the results is open to question.

(IV) McCarthy et al. (1958b) perfused the isolated goat liver with labelled fatty acids in the perfusate and measured the amount of isotope appearing in the carbohydrate (blood lactate plus glucose plus liver glycogen). Acetate, propionate and butyrate were used. The figures for recovery of the isotope in carbohydrate after one hour were 8%, 37% and 73% respectively. (It should be pointed out only 50% of C^{14} administered as propionate was found in toto at the end of the experiment; figures for acetate and butyrate were 72 and 90% respectively.) The fraction by weight of the total carbohydrate synthesized from administered fatty acid was small, approximately 2% for acetate, 8% for propionate and 1.7% for butyrate. Thus the liver relied predominantly on endogenous substrates for the synthesis of carbohydrate, and it is uncertain how far such figures apply to the intact animal. It should be pointed out that the finding of C^{14} in carbohydrate from a labelled fatty acid is not indicative of a net synthesis from the fatty acid. Acetate, for instance, is known to be metabolized extensively through the tricarboxylic acid cycle where it condenses with oxalacetate to form citrate (Fig. 1 p.114). This can be converted to pyruvate and hence by steps which are a modification of the glycolytic steps, to glucose (Krebs, 1954). But it appears that oxalacetate arises from carbohydrate or precursors of carbohydrate. So the carbon atoms of acetate appear in glucose only by this mechanism at the expense of carbon from known precursors of glucose.

(V) Kleiber and his associates (see Kleiber, 1954) injected C^{14} labelled fatty acids into cows and measured the isotope in various fractions of milk secreted over the next 48 hours. They showed that at least 80% of milk lactose arises from blood glucose. Thus, the amount of labelling of milk lactose indicates the relative contribution of the fatty acids for synthesis of glucose. Values inferred from the results of Kleiber and his colleagues are shown in Table 1; when labelled carbon is put in different positions in the fatty acid molecule, different amounts of isotope appear in the milk lactose.

This result would be expected if the isotope is incorporated into lactose by 'exchange', as has just been described for acetate (Weinman et al., 1957). Kleiber's data show that the ratio

$$\frac{\%\text{ isotope incorporated from beta-methyl carbon}}{\%\text{ isotope incorporated from carboxyl carbon}}$$

is smallest for propionate. This may be because for some propionate at least, the 3-carbon skeleton is incorporated intact into the glucose molecule. Thus in the classical concept of net synthesis of carbohydrate from fat, propionate is more glucogenic than the other two acids. If the amount of unlabelled fatty acid with which the injected isotope was in equilibrium was known, the exact contribution by weight of the three fatty acids to the lactose produced in that time could be estimated. Unfortunately this cannot be done, as there is a continual influx of the acids

from the rumen mixing with the injected pool. Also it is uncertain how long this mixing lasts.

Table 1.

Recovery of radioactivity in cow's milk following intravenous injection of labelled fatty acids (data of Kleiber, 1954)

Labelled isotope administered	% injected that appears in milk lactose
carboxyl-labelled acetate $CH_3C^{14}OOH$	1.4
methyl-labelled acetate $C^{14}H_3COOH$	4.6
carboxyl-labelled propionate $CH_3CH_2C^{14}OOH$	5.6
beta-C-labelled propionate $CH_3C^{14}H_2COOH$	10.2
carboxyl-labelled butyrate $CH_3CH_2CH_2C^{14}OOH$	2.7
beta-C-labelled butyrate $CH_3CH_2C^{14}H_2COOH$	6.1

The data have been standardized for an average milk yield of 20 kg. over the 46 hours of the experiment

Although results obtained by this technique must be regarded as inconclusive they do show that carbon from all fatty acids may make some contribution to carbohydrate by exchange, and suggest that propionate may be more glucogenic in a classical sense than either acetate or butyrate.

The distinction between exchange participation and net synthesis may be thought artificial; but it is of practical significance, in that acetate for instance can give rise to carbohydrate only when plenty of carbohydrate is available from other sources. When carbohydrate is limited the amount of carbon contributed by acetate cannot be increased by increasing the supply of acetate, but only by allowing glucose to be metabolized by routes which acetate would have taken.

From the evidence, particularly that of Johnson (1955) and Armstrong & Blaxter (1957), there is no reason to doubt that propionate is a significant source of carbohydrate. Equally, there has been generai acceptance of the thesis that acetate is not a source of carbohydrate in the sense that propionate is. Butyrate however has caused much controversy. It is possible that there may in some circumstances be net synthesis of

carbohydrate from butyrate, but even if this is found to be so its practical significance is uncertain. Although there is evidence that appreciable amounts are produced in the rumen, the amount available to the animal seems to be relatively small. Further work on this point seems desirable. From figures available at present (p.107) it seems that even if there were complete conversion of butyrate to glucose, it would possibly not contribute more to the carbohydrate stores than for instance, glucose coming from the gastro-intestinal tract. Incidentally, this conclusion applies with even more force to fatty acids higher than butyric, since the data of McCarthy et al. (1958a) suggest that the total amount produced by the rumen that is available to the animal is about 25% that of butyrate.

Sources (3). The sources of glucose so far considered supply about half the total daily glucose requirement. The only obvious source of the remainder is protein. Unfortunately it is difficult to estimate the amount of carbohydrate synthesized from this source. Kronfeld (1958) calculated that the urinary nitrogen in sheep on a good maintenance ration is about 9 g./day which corresponds to breakdown of 55 g. protein/day. Not more than 50–60 g. glucose could be produced from this. Kronfeld recognized the uncertainty of the calculation, and this may be enhanced, particularly in ruminants, where appreciable amounts of urea are produced from rumen ammonia (McDonald, 1948). In sheep on a good quality diet (800 g. hay plus 50 g. casein) Lewis et al. (1957) calculated that about 14 g. NH_3 nitrogen is carried to the liver over 24 hours. A variable fraction of this nitrogen returns to the rumen via salivary urea, and equally a variable fraction of urea arising from endogenous protein production may enter the rumen via saliva, rather than appear in the urine. Thus, an estimate such as the one above must be considered very tentative. However the estimate of glucose obtained by gluconeogenesis approximately equals the glucose requirement as suggested by studies of glucose turnover.

The blood sugar of ruminants. It has long been known that the blood sugar level in mature ruminants is generally lower than in man and laboratory animals. Reid (1950a) emphasized that this is equally true when blood sugar concentrations are measured by methods which have a high specificity for glucose. In unweaned ruminants the blood sugar level is similar to that of mature non-ruminants, and falls in the first few weeks or months. The exact time is uncertain. McCandless & Dye (1950) correlated the decline with the development of the rumen, but Reid (1953) claims that in lambs at least, the fall is often completed before rumination has begun. Part of the fall is due to loss of glucose from red cells but this does not entirely account for the fall in blood sugar with increasing maturity. As Widdas (1955) has shown, this change in permeability of the erythrocyte with increasing maturity is found in many types of animal as well as ruminants.

Fate of glucose produced.

Formation of glycogen. In a sense glycogen production need not be regarded as some fraction of the total glucose utilized; glycogen may fairly be regarded as an extension of blood glucose (that is, glucose in

transit from the liver to a terminal organ). It is probable that glycogen in both liver and muscle is used as a reserve; but even if for instance, it were shown that in muscle initial transformation of glucose to glycogen was necessary before it was catabolized, this would not affect the argument.

Thus, except in special circumstances, such as after a period of starvation, or during growth, account need not be taken of glycogen synthesis in assessing the total glucose requirements of an animal.

Glucose as an energy source. Reid (1950b) was one of the earliest workers to examine this problem. He estimated the uptake of glucose by the tissues of the head in sheep by measuring the blood glucose carotid–jugular difference. He pointed out that muscle constituted most of the metabolically active tissues thus studied. A–V differences obtained (1.5–2.0 mg./100 ml.) were constantly less than femoral A–V differences in the dog (5–19 mg.%) and man (70 out of 100 samples had A–V difference greater than 4 mg./100 ml. in the post-absorptive state (Somogyi, 1948)). For volatile fatty acids the position was reversed (sheep 0.5–4.0 mg.%, dog 0.1–0.2). Reid suggested that while glucose was the major source of energy for the dog, it was less important for the sheep, this function being partly taken over by acetate.

This assessment of acetate as a source of energy is supported by the work of Annison & Lindsay (1958). By continuous intravenous infusion of labelled acetate they showed that in sheep, acetate oxidation could account for 25% of the expired CO_2. The contribution of glucose was estimated by Baxter et al. (1955). Isotopically labelled glucose was injected into lactating cows and by following the decline of radioactivity in plasma glucose and its appearance in expired CO_2 they calculated that about 10% of the expired CO_2 originated from glucose. This is to be compared with values of 45–60% published by other workers for the rat and the dog.

However, earlier estimates of this type, obtained in non-ruminants, have been criticized. Searle et al. (1956), using the technique of constant infusion of labelled material, found that in the dog only 25% of the expired CO_2 originates from glucose. Baker et al. (1954) give a figure of 20% for man. Indeed the whole concept that carbohydrate is the main immediate energy source in animals has become less tenable. Thus Andres et al. (1956) found that in man, glucose oxidation in muscle (probably half the body weight) could account for no more than 7% of the CO_2 expired. In non-ruminants evidence has been accumulating that fat is extensively used as a source of energy. Fritz et al. (1958) have shown that fatty acids can be directly oxidized by muscle, and evidence reviewed by Frederickson & Gordon (1958) suggests that in man the non-esterified fatty acids in plasma are the source of a substantial fraction of the expired CO_2. These acids are long-chain fatty acids such as palmitic and oleic. They are found in ruminant blood in addition to the short-chain acids (Lindsay, unpublished) although their contribution to ruminant metabolism is as yet uncertain.

It should be pointed out that the estimation of glucose contribution to CO_2 as studied in non-ruminants was based on relatively short-term periods (less than 8 hours). The estimate of Baxter et al. in cattle was

over 48 hours. Short-term experiments probably mainly estimate glucose directly oxidized to CO_2, whereas the estimate of Baxter et al. would include glucose converted to some other component (e.g. fat) which was later oxidized. It is likely however (p.118) that while body fat is extensively synthesized from glucose in non-ruminants, this is probably not so in ruminants, acetate being used instead.

Thus Reid's original concept must be modified. In non-ruminants glucose entering from the gastro-intestinal tract is partly oxidized but the bulk is converted to body fat to be oxidized later. In ruminants part of the acetate provided by the diet will be oxidized directly, while much of the rest will be converted to body fat for later oxidation.

Inter-relationship of glucose and acetate oxidation. The evidence suggests that loss of glucose by oxidation is kept to a minimum. Modern biochemical theory suggests that some oxidation of carbohydrate is inevitable when acetate is oxidized (Deuel, 1957). An outline of the steps leading to the oxidation of glucose or acetate is shown in Figure 1.

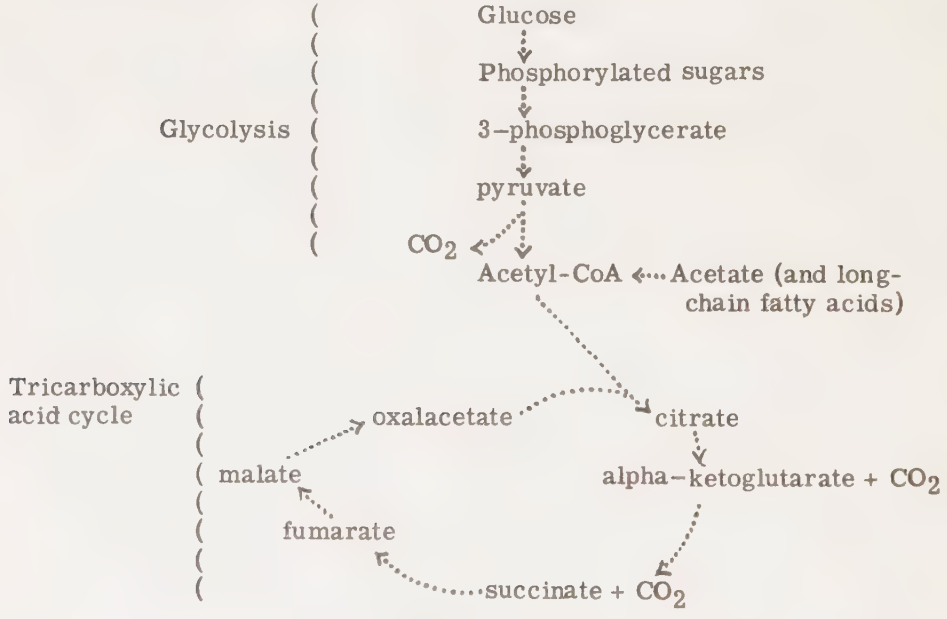

Figure 1.

In both, the carbon atoms are ultimately converted to CO_2 through the tricarboxylic acid cycle. Oxidation of glucose or acetate requires the presence of adequate amounts of the members of this cycle (oxalacetate, citrate etc.). As was pointed out above (p.110) the weight of evidence suggests that in animals these substances arise only from carbohydrate or carbohydrate precursors. The concept has frequently been expressed that acetate is oxidized only in the presence of carbohydrate. But the

tricarboxylic acids are catalysts. The amount of degradation of carbohydrate required to permit oxidation of acetate depends on the rate of loss of these acids from the cycle. Part of this loss probably occurs because oxalacetic acid is unstable and tends to break down slowly to produce pyruvate and CO_2; but in liver for instance, probably much more important would be the removal of cycle acids for biosyntheses.

In ruminants the utilization of acetate depends on that of glucose as was demonstrated by Jarrett & Potter (1957); and Reid (1958), who explained it by the mechanism outlined above. This mechanism has been widely used to account for the development of ketosis during a shortage of carbohydrate (Beatty & West, 1951; Ho & Reber, 1957b). It is supposed that in such conditions the supply of the tricarboxylic acids (oxalacetate in particular) is insufficient to permit the oxidation of acetate. Acetyl-coenzyme A tends to accumulate and is then converted to beta-hydroxy butyric acid which appears in the blood. Recent work however, does not support this; Lindsay & Ford (in preparation), by administering labelled acetate, found that acetate oxidation is not affected when ketosis is induced in sheep in late pregnancy by reducing the diet. In addition, by comparing the $C^{14}O_2$ expired when either methyl- or carboxyl-labelled acetate was used, they estimated the supply of precursors of tricarboxylic acids. (Weinman et al., 1957 give details of this technique.) No diminution was observed during the ketosis. But it is possible that there was a decrease specifically in the liver. Bach & Hibbitt (1959) showed that in bovine ketosis there is a fall in the plasma succinate and citrate and they inferred decreased levels in tissues. However, Kalnitzky & Tapley (1958) and Shaw & Tapley (1958) measured directly the oxalacetate in rat liver and found no fall during fasting ketosis or alloxan diabetic ketosis. It would clearly be desirable to confirm this in ruminant ketosis. The suggestion that shortage of carbohydrate may be a limiting factor for the oxidation of acetate in ruminants cannot as yet be regarded as established.

Significance of oxidative energy. Energy liberated by the oxidation of carbohydrate has been generally accepted as available for heat, for biosyntheses, and for muscular activity (e.g. Baldwin, 1952). Since, from the evidence adduced above, acetate to some extent replaces glucose for this purpose in ruminants, it may be asked whether the energy obtained by oxidation of acetate is equally available.

That increased heat production follows utilization of acetate is well established (Armstrong & Blaxter, 1957). However, Reid (1950b) referred to acetate, at least in some circumstances, as being "rapidly oxidized without providing useful energy in the productive channels of metabolism". This possibility was also raised by Phillipson & Cuthbertson (1956), who stated "the physiological question that still requires an answer is whether the energy of acetate can be manifested as muscular activity".

There is no reason to doubt this. Most of the energy generated by the oxidation of glucose, as of acetate, arises during oxidation through the tricarboxylic acid cycle. Presumably energy will be produced in a similar way from both substances.

Lifson et al. (1951) observed increased oxidation of labelled acetate by the isolated perfused dog gastrocnemius during contraction. Nakamura

et al. (1949) showed that the beat of the rat heart depleted of substrate was revived by addition of acetate, and Furchgott & Shorr (1946) reported similar results for the contraction of intestinal smooth muscle.

No comparable studies appear to have been made with ruminant tissues, but it seems improbable that they are less well adapted to utilize acetate than those of laboratory animals.

Synthesis of fat from glucose. Stetten & Boxer (1944) first showed in rats given a diet rich in carbohydrate that this is one of the major pathways by which glucose is utilized; up to 60% of the glucose utilized was converted to fat. In ruminants similar studies in the whole animal have not been made, but considerable study has been made of milk fat synthesis. Early work indicated that the lactating udder in vivo has a respiratory quotient (R. Q) greater than unity (Graham et al., 1938) which suggested that the udder synthesizes some at least of its milk fat from carbohydrate. However, Folley & French (1949) showed that while lactating mammary gland slices of laboratory animals utilized glucose with RQ greater than 1, slices from the ruminant mammary glands (goat and cow) did not and in its presence RQ was less than 1. A RQ greater than 1 was obtained however in the presence of acetate with ruminant tissue but not with non-ruminant slices, except for the rabbit (Folley & French, 1950). The participation of acetate in milk fat synthesis was more directly shown by Balmain et al. (1954), who, using labelled acetate, found that sheep mammary slices incorporated glucose into fatty acids at only about one thirtieth the rate at which acetate was incorporated. Evidence that this holds in vivo was provided by Rogers & Kleiber (1956). In lactating cows nearly 10% of labelled acetate but only 1% of labelled glucose was found in the milk fatty acids.

This does not mean that only acetate or glucose are used for synthesis of milk fatty acids. Glascock (1958) drew attention to the significance of blood lipids for milk fat synthesis in cattle. However as far as small molecules are used for synthesis of milk fatty acids it does appear that in ruminants the place of glucose is taken over by acetate. Although the other fatty acids can participate in fat synthesis, they have not been considered since they are normally almost absent from peripheral blood; their participation probably depends on initial transformations in the liver.

While the evidence suggests that there need be no glucose drain for milk fatty acid synthesis, this probably does not hold for milk fat, which is largely esterified. Glucose will thus probably be required for glycerol synthesis. Conventional biochemical opinion regards glycerol as glycogenic (only carbohydrate or carbohydrate precursors lead to net synthesis of glycerol; acetate will only contribute to glycerol carbon by exchange (Deuel, 1957)). For glycerol synthesis in non-ruminants the work of Popjak et al. (1953) is consistent with this view. They injected labelled glucose into lactating rabbits. The specific radioactivity of milk glycerol isolated after labelled glucose was given was very similar to that of lactose; French et al. (1952) showed that after administration of labelled starch to rabbits by stomach-tube, lactose and glucose have identical specific activities. When labelled acetate was injected, much less of the glycerol was labelled, although the specific activities of blood glucose and

milk glycerol were very similar. Thus, acetate is not important as a source of milk glycerol. What fraction of labelled acetate does get there arises indirectly from the small amount of acetate converted to blood glucose by "exchange labelling".

It is uncertain how far these observations apply to ruminants. Dimant et al. (1953) found in the isolated bovine udder perfused with labelled glucose that milk lactose became labelled but the glycerol of fat did not. This may be because the glycerol is not synthesized in the udder; observations of Rogers & Kleiber (1956) suggest that in the lactating cow glucose is a much more important source of glycerol than the fatty acids. Thus, some glucose is required to supply the glycerol of milk fat but the amount is difficult to assess.

There is another more subtle function of glucose, namely to supply cofactors essential for fatty acid synthesis. Detailed biochemical knowledge of fatty acid synthesis is incomplete, but considerable work has been done (e.g. Langdon, 1957; Wakil & Ganguly, 1959; Hele & Popják, 1958). It is agreed that for the synthesis of fatty acids the presence of reduced triphosphopyridinenucleotide (TPNH) is required. There are few biochemical systems in which reduced TPN is generated; but one of them is what is known as the 'indirect' or pentose phosphate oxidation of glucose (Glock & McLean, 1958). The overall reaction is: 3 glucose - 6 - phosphate + 6 TPN→ 3 CO_2 + 6 TPNH + glyceraldehyde - 3 - phosphate + 2 glucose - 6 - phosphate. Glock & McLean (1953) showed that the activities of the two enzymes which produce reduced TPN are higher in rat mammary gland at the height of lactation than in any other animal tissue they have investigated. McLean (1958b) found that the level of TPNH in rat mammary tissue is low in pregnancy, rises nearly a hundredfold in lactation, and falls sharply at the end of lactation. This pathway is extremely important in the lactating mammary gland of the rat, and it is tempting to suppose that one important function is the provision of TPNH for fat synthesis.

In ruminants, the significance of this pathway has not been demonstrated so strikingly. McLean (1958a) showed that the activity of the enzymes producing TPNH is high in the sheep mammary gland during lactation. The activity did not increase markedly as lactation progressed, but peak milk secretion was reached earlier in the sheep than in the rat. Evidence of Black et al. (1957) is consistent with the view that the pathway of oxidation of pentose phosphate is important in the lactating cow. The amount of glucose metabolized by this pathway is unknown.

So far, the possible glucose requirement for milk fat synthesis has been considered. Milk fat however, differs from body fat in the cow. Cow's milk fat contains about 10% of short-chain acids (C_4–C_{12}) whereas ox depot fat consists mainly of long-chain acids (stearic, palmitic and oleic) (Hilditch, 1947).

Recently, Lindsay (in preparation) obtained indirect evidence that similar considerations may hold for depot fat, in the sheep. The existence of non-esterified long-chain fatty acids (NEFA) in ruminant blood has been noted earlier (p.113). Studies in non-ruminants suggest that these acids originate from adipose tissue, a rise in blood levels indicating breakdown of fat depots (Frederickson & Gordon, 1958). The

raised levels found on fasting are rapidly brought down by administration of carbohydrate. In sheep the level is brought down equally efficiently by administration of acetate. It would appear however that some glucose utilization is required. Just as in diabetes in man, the high levels of NEFA are only slightly reduced by giving glucose, the high levels found in sheep in late pregnancy are not much affected by administration of acetate (Annison, personal communication). This glucose requirement for synthesis of depot fat from acetate could be either for glycerol or TPNH synthesis, as described above for milk fat.

Further investigation of this is required; but it seems reasonable to suggest that, while some glucose is required in fat synthesis, the extensive participation found in non-ruminants probably does not occur in ruminants.

Glucose requirement for special purposes. Some glucose or carbohydrate equivalent is probably required for a number of special purposes, e.g. in connective tissue, glycolipids in brain, ribose in nucleic acids etc. It is impossible to estimate this requirement, but it is presumably small compared to the total glucose turnover, and there is no evidence that ruminants have any special requirement or adaptations to meet this need.

Two functions that merit consideration are the demands of the foetus in pregnancy, and requirement for milk lactose in lactation.

Demands of pregnancy. This requirement has been recently estimated by Kronfeld (1958). Glucose is required per se and also for the synthesis of fructose. The estimates were based mainly on data published by Huggett and his colleagues (Huggett et al., 1951; Cooper et al., 1949; Hitchcock, 1949) of the average umbilical A–V difference and flow rate. The daily fructose requirement was 3–7 g. at 100 days' pregnancy, and 13–17 g. at term. The glucose uptake was independent of the maternal blood sugar level, and was regarded as an obligatory drain. For glucose the uptake was 3–9 g./day at 100 days, and 12–22 g./day at term and depended on the relative blood sugar concentrations of maternal and foetal blood. These figures are probably true for a relatively high maternal blood sugar level. They would be increased when twin or triplet foetuses were present. Foetal requirements therefore account for a substantial fraction of the total glucose turnover.

In contrast uptake of acetate at term was about 6 g./day. Comparable data are not available for other ruminants, but since fructose in the blood is a characteristic of new-born ruminants and other ungulates (Goodwin, 1956) similar considerations probably apply.

Demands of lactation. The demands of lactation are even greater. In the cow milk production of about 14 litres/day is a reasonable average (Milk Records, 1958). With an average milk lactose of 4.9% about 700 g. glucose would be required simply for milk lactose which is over 50% of the glucose turnover in lactating cattle (p.108). In the sheep, the data of Godden & Puddy (1935) and earlier workers, suggest 1 litre/day as a reasonable milk yield with a lactose content of 4.6–5.4%. The glucose requirement for lactose of about 50 g./day is about half the total glucose turnover. Peirce (1934) found in 6 Merino sheep after 9 weeks of

lactation the milk yield averaged 650 g./day. Estimates in the goat are the most striking of all. Milk Records (1958) show the average milk yield of goats in East England is about 5 litres/day. If the lactose content is 4% this alone requires 200 g. glucose a day. The glucose turnover must therefore be much higher than earlier estimates in this review; such a figure could only be obtained by a very high standard of animal husbandry.

It seems clear that in ruminants, lactation and pregnancy are the physiological functions requiring most of the glucose.

Other monosaccharides.

(1) Fructose metabolism was studied in the lactating cow by Luick et al. (1957) by injection of labelled fructose. Within 15 min. of injection labelled glucose began to appear in the blood, suggesting rapid and efficient conversion to glucose. The fructose radioactivity declined more rapidly than after injection of labelled glucose and from this and observation of the radioactivity in CO_2 and milk the authors suggested that (a) up to 50% of injected fructose appears in CO_2 and milk by pathways which do not necessitate previous conversion to glucose (b) fructose is probably metabolized by extra-hepatic tissues. Since fructose may occur in small amounts in bovine blood (Rice, 1953), the authors suggest fructose metabolism may be more important than has been generally recognized.

(2) Galactose. The rate of clearance of injected galactose was studied in cattle by Garner (1952), who found that it is considerably slower than in man. And since it was slower after severe liver damage, Garner considered it might have value as a test of liver function. But this is doubtful because he found that with mild liver damage the clearance rate was increased.

(3) Lactose. Since galactose is not detectable in mammalian blood, milk lactose must be synthesized in the mammary gland itself. Much work has been done on the mechanism of synthesis of lactose in the udder. It is not considered further here since it has been adequately reviewed by Malpress (1958).

Carbohydrate metabolism in individual organs.

Muscle. The studies by Reid (1950b), discussed earlier, of the biochemical activity of the tissues of the sheep's head, were interpreted as showing that ruminant muscle utilized acetate more than glucose. Because of the heterogeneity of the tissues of the head (the possible contribution of adipose tissue was ignored by Reid) the evidence is equivocal.

Heart. Pennington (1952) reported briefly on the uptake of volatile fatty acids by sheep heart slices; less than 1 μmol./g. wet wt./hour was taken up. This is very different from that of rat heart slices, quoted by Pearson et al. (1949) – acetate oxidation to CO_2 was about 35 μmol./g. wet wt./hour. Lindsay (unpublished) compared the isolated perfused rat heart and sheep heart. Uptake of acetate, at a circulating concentration of 2–6 mM/litre was 18 μmol./g. wet wt./hour for the sheep; 44 μmol. per g. wet wt./hour for the rat. Pennington's low value may be due more to

difficulty in preparation of the slices than to poor ability of the heart to utilize acetate. Glucose utilization figures in the perfused heart, at normal blood concentrations, and in the absence of insulin, were 100 μmol./g. wet wt./hour for the rat and 25 μmol./g. wet wt./hour for the sheep.

The lower figures for both glucose and acetate uptake in the sheep heart probably reflect the lower metabolic activity per unit mass of a larger animal, but on the whole they suggest no great metabolic differences between sheep and rat hearts.

Brain. McClymont & Setchell (1956) studied the uptake of metabolites by the sheep brain in vivo by sampling venous blood from the dorsal sagittal sinus using a permanent in-dwelling polythene catheter. The concentrations of acetate and glucose were compared with samples taken simultaneously from the carotid: the mean A–V difference for acetate was almost zero (range −0.4 to + 0.4 mg./100 ml.). For glucose the mean difference was 6.7 ± 0.7 mg./100 ml. The mean oxygen A–V difference was only slightly greater than would account for complete oxidation of the glucose taken up. As Kronfeld (1958) pointed out, the significance of this is uncertain, since the arterial blood was apparently only 50% saturated. The data nevertheless are consistent with the view that the sheep brain resembles that of non-ruminant animals in depending largely on glucose as an energy source.

Rumen epithelium. Pennington and his colleagues (e.g. Pennington & Sutherland, 1956; Pennington & Pfander, 1957) studied extensively the metabolism of sheep rumen epithelium in vitro. They found appreciable metabolism of the volatile fatty acids, particularly propionate and butyrate. Propionate was readily converted to lactate via succinate. Butyrate, and to a minor extent acetate, gave rise to ketone bodies. Glucose uptake was about 7.5 μmol./g. wet wt./hour and not greatly affected by the presence of volatile fatty acids. The utilization of fatty acids (particularly acetate) was increased by the presence of glucose, but there was still substantial conversion of butyrate to ketone bodies. Pennington considered that much of the butyrate produced in the rumen is converted by epithelial tissue to ketone bodies, and this view was supported by Annison et al. (1957), who showed in vivo that little butyrate is found in the portal vein of sheep, but the level of ketone bodies is higher there than in peripheral blood. McCarthy, Shaw, McCarthy, Lakshmanan, & Holter (1958a) challenged this view. In the isolated perfused rumen they were unable to find an increase in ketone bodies in the perfusate, whereas they found an increase in free butyrate. It is impossible to decide which view is correct, but the evidence of Annison et al. is stronger, since it was obtained under more physiological conditions. It is conceivable that in the experiments of McCarthy et al. (1958a) the rumen epithelium was more anoxic, when ketone bodies may be produced less readily.

Enzymes involved in carbohydrate metabolism. The only reference dealing specifically with ruminants appears to be that of Jarrett & Filsell (1958), who examined the hexokinase activity of sheep brain and intestinal homogenates and compared the results with those in rats. The activity of intestinal

homogenates was only 30% of that in rats, except for lambs (12 hours to 5 days old) where it was about 50%. In the sheep brain, activity was about 50% that of the rat. It is difficult to assess these results, since the activity/unit weight of enzymes concerned with energy metabolism, such as hexokinase, might be higher in a small animal such as the rat.

Setchell (1959) found the hexokinase activity of sheep brain to be only slightly lower than that of guinea-pig brain. The results cannot strictly be compared with those of Jarrett & Filsell, but the values for sheep brain are probably of the same order.

McLean (1958a) recorded the activities of glucose-6-phosphate dehydrogenase and of 6-phospho-glucose-dehydrogenase in the udder and liver of the lactating ewe. As might be expected, they were less than those in the rat; but did not differ greatly from those in the rabbit.

Hormones concerned with carbohydrate metabolism.

Insulin. There are several reports of the effect of insulin in sheep. The most complete are those of Reid (1951a,b; 1952), who showed that in normal sheep injection of insulin lowers the blood sugar, but that the rate of fall is much slower than is usually observed in non-ruminants. The effect is similar to that observed in rats, mice and rabbits on a high fat diet (Bainbridge, 1925; Himsworth, 1934). The rate of fall was greatest when 1 unit/kg. of insulin was administered. Larger doses (up to 10 U/kg.) merely prolonged the hypoglycaemia. Other striking features were the tendency of the blood sugar to remain above 5 mg./100 ml., and the resistance of the animals to hypoglycaemic convulsions. Some neurological signs always occurred with blood sugar levels below 5 mg./100ml. but convulsions were observed only when the blood sugar had been below 1 mg./100 ml. for at least one hour. Reid suggested that in the development of neurological signs at low blood sugar levels, the sheep does not differ from laboratory animals. The occurrence of symptoms at higher blood sugar levels in the rat, for example, is due to reducing substances in the blood, which are measured as glucose by many tests for blood sugar.

The other interesting effect was the negligible change in the glucose A-V difference between carotid and jugular blood after injection of insulin. When insulin is injected in man or the rabbit, the A-V difference for tissues of the arm or hind-limb generally increases, sometimes to as much as 20-30 mg./100 ml. (Friedenson et al., 1928, Cori & Cori, 1925).

The contrast between the sheep and man or the rabbit must be treated with reserve since (a) the A-V differences relate to different parts of the body (b) no information was obtained on the blood flow; however, the data do suggest that the tissues of the sheep are more resistant to insulin than those of man and the dog. In this connexion unpublished data of Lindsay are of interest. While the glucose utilization of the isolated perfused rat heart increased up to fourfold in the presence of insulin, only a small increase was observed in the perfused sheep heart. But the response of the sheep heart was more varied; occasionally big effects were obtained. From this and the observation of Reid, it is probable that sheep muscle responds less to insulin than rat, rabbit or

human muscle.

When insulin was injected in the cow (Jasper, 1953) the rate of fall of the blood sugar, although a little more rapid than in the sheep, was slower than in non-ruminants.

Diabetes in ruminants. Spontaneous diabetes is uncommon in ruminants; Wilkinson (1957) listed the cases in the literature. Lukens (1938) observed that pancreatectomy in the goat was followed by mild diabetes and similar results were reported by other workers in sheep and calves (Blombäck et al., 1953; Cook et al., 1949). The blood sugar was often high, but ketosis was absent, and the animals remained well for some months without treatment with insulin. These observations and the results quoted above suggest that insulin has less effect in ruminants than non-ruminants. However, Jarrett (1946) and Innes (1950) observed severe diabetes in sheep after injection of alloxan. Jarrett et al. (1956) studied in more detail the effect of pancreatectomy in sheep. In some animals the effect was similar to that seen in goats by Lukens, but severe diabetes with marked ketosis occurred in 3 of 11 which survived operation.

Finally, Cunningham (personal communication) measured plasma insulin levels in the cow, and reported values of 0.1–2 mU/ml. plasma. These are of the same order as figures reported in man (Groen et al., 1952) and in the cat and rat (Randle & Young, 1956).

Evidence on the significance of insulin in the carbohydrate metabolism of ruminants is confusing. This may be partly due to variation of the diet in different conditions. It is well known for instance that the clearance of injected glucose is much slower in ruminants than other mammals (e.g. Holmes, 1951). In part at least, this could be due to a diminished output of endogenous insulin in response to injected glucose, such as is known to occur in the rat (Anderson & Long, 1947). The sluggish response could simply be due to the ruminant diet which is rich in fat (in terms of substances actually entering the circulation). In support of this hypothesis, Reid (1958) showed that sheep eliminated injected glucose almost as fast as man when the diet contained a high proportion of cracked maize. Such a diet would increase the amount of propionate (and hence carbohydrate) available to the animal.

However, this is not the whole explanation; an animal factor is implicated as well. Reid found differences in the clearance of injected glucose between sheep on the same diet. The data of Jarrett et al. (1956), quoted above, in which there appeared to be two types of response to pancreatectomy in sheep, can hardly be explained by dietary differences.

Clearly, further work is desirable on the nature of the action of insulin in ruminants.

Adrenaline. The hyperglycaemia which occurs in laboratory animals after injection of adrenaline also occurs in sheep (Setchell & McClymont, 1955) and cows (Garner, 1952). It is highly probable that this effect of adrenaline is of physiological importance since the tendency of sheep to maintain a blood sugar above 5 mg./100 ml. after injection of insulin, is lost after splanchnic section (Potter, 1952) or adrenalectomy (Reid, 1948).

Glucagon. Ho & Reber (1957) found that injection of 1–2 μg./kg. raised

the blood sugar of ewes for up to 4 hours. A similar effect was found when the initial blood sugar was low (12–20 mg./100 ml.) in ewes fasted 5–7 days in the last third of pregnancy.

Growth hormone. Kronfeld (1957) described the effect of intramuscular injection of growth hormone in ewes in the last month of pregnancy. The blood sugar rose by about 12 mg./100 ml., the higher level persisting 5 days, but returned to the pre-injection level despite continued administration of the hormone. There was also a tendency for blood ketone bodies to rise but this was less positive. Thus, in sheep, growth hormone does not appear to be diabetogenic as it is in cats and dogs (Cotes et al., 1949) and only slightly ketogenic compared to the effect in the rat (Lotspeich & Petersen, 1954).

ACTH and cortisone. Setchell & McClymont (1955) found that, in pregnant ewes, the injection of cortisone (200 mg.) or of long acting ACTH (40 U) produced a sustained hyperglycaemia for at least 24 hours. Similar effects along with a decline in milk yield have been observed in lactating cattle (Shaw, 1955). If growth hormone was given with ACTH, the rise in blood sugar and decline in milk yield did not occur.

Conclusion and general significance. The object of this review, as was stated at the beginning, was to examine the view that the metabolism of glucose is quantitatively less important in ruminants than in other animals. On the present evidence this is unacceptable. It is true however, that ruminants use glucose differently owing to the special characteristics of the rumen. The carbohydrates of the diet, instead of being broken down to glucose which is directly available to the animal, are fermented largely to the volatile fatty acids with some loss of carbon in rumen gas. Of these fatty acids only a portion can be converted by the animal, to lead to a net synthesis of carbohydrate. Ruminants would appear to have modified their needs for carbohydrate to meet these conditions. In particular, less glucose is oxidized, and probably much less is used for synthesis of fat, the carbohydrate thereby saved being available for the requirements of pregnancy and lactation. At the same time the use of acetate for oxidation and fat. synthesis is limited ultimately by the need for some simultaneous utilization of carbohydrate. The carbohydrate economy of ruminants is therefore precarious — changes either in the quality of the diet or in the demands for carbohydrate can readily alter the carbohydrate balance from the normal to the pathological.

It is highly probable that this is the basis of some of the metabolic disorders of ruminants, e.g. pregnancy toxaemia in the ewe and acetonaemia in the cow. The most consistent biochemical signs are low blood sugar and increased blood ketones. The usual explanation of the aetiology is that there is insufficient carbohydrate for the oxidation of acetate, hence the liver responds by converting acetate to ketones which then appear in the blood. It is equally possible that fat synthesis from acetate is diminished and that the fat depots release long-chain fatty acids which overload the liver and cause ketosis. Whatever the explanation

there is little doubt that the utilization of carbohydrate is interfered with. Many of the biochemical deviations in these diseases — the elevated blood ketone and long-chain fatty acids, the fatty liver, and the decreased acetylating ability of the liver (Peeters et al., 1958) — are reminiscent of diabetes mellitus in man. It is reasonable to suppose that the disturbance in carbohydrate metabolism is a primary factor in the development of these diseases, since in ketosis for instance, administration of glucose or substances which raise the blood sugar is a highly successful treatment. The lack of success of the use of such substances in the treatment of pregnancy toxaemia was attributed by McClymont & Setchell (1955) to irreversible cerebral damage due to a sustained hypoglycaemia before treatment began. However, even if "disturbance in carbohydrate metabolism" is a major factor, the reason for the disturbance is not clear, and may be different in the two cases. The poor understanding of the origins of these conditions probably reflects a dearth of fundamental knowledge; evidence shows for instance how inadequate is information on endocrine control of carbohydrate metabolism in ruminants.

Nevertheless, the information available may be of value in assessing some possibilities. It is sometimes suggested that pregnancy toxaemia is characteristic of sheep and acetonaemia of cattle, because the greater drain of glucose is in pregnancy in the sheep and in lactation in the cow. The situation must be more complex than this; even in sheep lactation probably requires more carbohydrate than pregnancy and in goats the drain of lactation is greater than in cows, yet acetonaemia is less frequent in the goat.

Although their metabolism of carbohydrate is finely balanced, ruminants are well adapted to their diet. Attempts to vary the diet so as to simulate that of a non-ruminant may have unexpected repercussions. Readily fermentable carbohydrate or well chopped hay would increase the production of propionic acid in the rumen at the expense of acetic (Balch & Rowland, 1957) and make more glucose available to the animal. This may make the glucose "bank balance" look more healthy, but may lead to a sharp fall in the milk fat. The precise mechanism behind this is not known, but almost certainly reflects the preference of ruminants to use acetate rather than glucose for synthesis of fat.

Considerations of this kind suggest caution in extrapolating to ruminants information about carbohydrate metabolism in laboratory animals, and such considerations provide the chief justification of this review.

REFERENCES

ANDERSON, E. & LONG, J. A. (1947). Endocrinology 40, 92.
ANDRES, R., CADER, G. & ZIERLER, K. L. (1956). J. clin. Invest. 35, 671.
ANNISON, E. F. (1954a). Biochem. J. 57, 400.
ANNISON, E. F. (1954b). Biochem. J. 58, 670.
ANNISON, E. F. (Personal communication.) Faculty of Rural Science, Armidale, N.S.W., Australia.
ANNISON, E. F., HILL, K. J. & LEWIS, D. (1957). Biochem. J. 66, 592.
ANNISON, E. F. & LEWIS, D. (1959). "Metabolism in the rumen." London:

Methuen.
ANNISON, E.F., LEWIS, D. & LINDSAY, D.B. (1959). J. agric. Sci. In press.
ANNISON, E. F. & LINDSAY, D. B. (1958). Biochem. J. 69, 33P.
ARMSTRONG, D. G. & BLAXTER, K. L. (1957). Brit. J. Nutr. 11, 247.
ASH, R. W., PENNINGTON, R. J. & REID, R. S. (1958). Biochem. J. 71, 9P.
BACH, S. J. & HIBBITT, K. G. (1959). Biochem. J. 72, 87.
BAINBRIDGE, J. W. (1925). J. Physiol. 60, 293.
BAKER, N., SHREEVE, W.W., SHIPLEY, R.A., INCEFY, G.E. & MILLER, M. (1954). J. biol. Chem. 211, 575.
BALCH, D. A. (1958). Brit. J. Nutr. 12, 18.
BALCH, D. A., & ROWLAND, S. J. (1957). Brit. J. Nutr. 11, 288.
BALDWIN, E. (1952). "Dynamic Aspects of Biochemistry." Cambridge University Press. (2nd edition.)
BALMAIN, J. H., FOLLEY, S. J. & GLASCOCK, R. F. (1954). Biochem. J. 56, 234.
BARCROFT, J. (1945). Proc. Nutr. Soc. 3, 247.
BARCROFT, J., McANALLY, R.A. & PHILLIPSON, A.T. (1944). J. exp. Biol. 20, 120.
BARHYDT, J. B. & DYE, J. A. (1957). Cornell Vet. 47, 76.
BAXTER, C. F., KLEIBER, M. & BLACK, A. L. (1955). Biochim. Biophys. Acta 17, 354.
BEATTY, C. H. & WEST, E. S. (1951). J. biol. Chem. 190, 603.
BENTLEY, O.G., LEHMKUHL, A., JOHNSON, R.R., HERSHBERGER, T.U. & MOXON, A. L. (1954). J. Amer. chem. Soc. 76, 5000.
BLACK, A. L., KLEIBER, M., BUTTERWORTH, E. M., BRUBACHER, G. B. & KANEKO, J. J. (1957). J. biol. Chem. 227, 537.
BLOMBÄCK, M., BLOMBÄCK, B., HOFLUND, S., JORPES, E., KALLNER, S. & MÅNSSON, J. (1953). Acta physiol. scand. 29, 170.
CARROLL, E. J. & HUNGATE, R. E. (1954). Appl. Microbiol. 2, 205.
CLARKE, R. & MALAN, J. R. (1956). Onderstepoort J. 27, 101.
CONRAD, H. E., WATTS, W. R., IACONO, J.M., KROYBILL, H.F. & FRIEDEMANN, T. E. (1958). Science 127, 1293.
CONRAD, H. R., SMITH, H. R., VANDERVELL, J. H., POUNDEN, W. D. & HIBBS, J. W. (1958). J. Dairy Sci. 41, 1094.
COOK, E. T., DYE, J. A. & McCANDLESS, E. L. (1949). Amer. J. Physiol. 156, 349.
COOPER, K. E., GREENFIELD, A. D. M., & HUGGETT, A. StG. (1949). J. Physiol. 108, 160.
CORI, C. F. & CORI, G. T. (1925). Amer. J. Physiol. 71, 688.
CORI, G.T., CORI, C.F. & BUCHWALD, K.W. (1930). J. biol. Chem. 86, 375.
COTES, P. M., REID, E. & YOUNG, F.G. (1949). Nature, Lond. 164, 209.
CUNNINGHAM, N. F. (Personal communication.) Central Veterinary Laboratory, Weybridge, Surrey.
DEUEL, H. J. (1957). "The lipids." Vol. 3. New York and London: Interscience publishers.
DIMANT E., SMITH, V. R. & LARDY, H.A. (1953). J. Biol. Chem. 201, 85.
ELSDEN, S. R. (1945). J. exp. Biol. 22, 51.
EL-SHAZLY, K. (1952). Biochem. J. 51, 640.
FEGLER, G. & HILL, K. J. (1958). Quart. J. exp. Physiol. 43, 189.
FOLLEY, S. J. & FRENCH, T. H. (1949). Biochem. J. 45, 117.

FOLLEY, S. J. & FRENCH, T. H. (1950). Biochem. J. 46, 465.
FREDERICKSON, D. S. & GORDON, R. S. (1958). Physiol. Rev. 38, 585.
FRENCH, T. H., POPJÁK, G. & MALPRESS, F. H. (1952). Nature, Lond. 169, 71.
FRIEDENSON, M., ROSENBAUM, M. K., THALHEIMER, E. J. & PETERS, J. P. (1928). J. biol. Chem. 80, 269.
FRITZ, I. B., DAVIS, D. G., HOLTROP, R. H. & DUNDEE, H. (1958). Amer. J. Physiol. 194, 379.
FURCHGOTT, R. F. & SHORR, E. (1946). Proc. Soc. exp. Biol., N.Y. 61, 280.
GARNER, R. J. (1952). J. comp. Path. 62, 292.
GLASCOCK, R. F. (1958). Proc. Roy. Soc. Ser. B. 149, 402.
GLOCK, G. E. & McLEAN, P. (1953). Biochim. Biophys. Acta 12, 590.
GLOCK, G. E. & McLEAN, P. (1958). Proc. Roy. Soc. Ser. B. 149, 354.
GODDEN, W. & PUDDY, C. A. (1935). J. Dairy Res. 6, 307.
GOODWIN, R. F. W. (1956). J. Physiol. 132, 146.
GRAHAM, W. R., HOUCHIN, O. B., PETERSON, V. E. & TURNER, C. W. (1938). Amer. J. Physiol. 122, 150.
GRAY, F. V. & PILGRIM, A. F. (1951). J. exp. Biol. 28, 83.
GROEN, J., KAMMINGA, C. E., WILLEBRANDS, A. E. & BLICKMAN, J. R. (1952). J. clin. Invest. 31, 97.
HALLIWELL, G. (1957). J. gen. Microbiol. 17, 153 & 166.
HALSE, K. & VELLE, W. (1956). Acta physiol. scand. 37, 380.
HEALD, P. J. (1952). Brit. J. Nutr. 5, 84.
HEALD, P. J. & OXFORD, A. E. (1953). Biochem. J. 53, 506.
HELE, P. & POPJÁK, G. (1958). Proc. Roy. Soc. Ser. B. 149, 414.
HESTRIN-LERNER, S. & SHAPIRO, B. (1954). Biochim. Biophys. Acta 13, 54.
HILDITCH, T. P. (1947). The chemical constitution of natural fats. 2nd edit. London: Chapman & Hall.
HIMSWORTH, H. P. (1934). J. Physiol. 81, 29.
HITCHCOCK, M. W. S. (1949). J. Physiol. 108, 117.
HO, P. & REBER, E. F. (1957a). Amer. J. vet. Res. 18, 342.
HO, P. & REBER, E. F. (1957b). Cornell Vet. 47, 131.
HOLMES, J. R. (1951). J. comp. Path. 61, 1.
HOLMES, J. R. (1952). J. comp. Path 62, 41.
HOWARD, B. H. (1955). Biochem. J. 60, p. i. of Proceedings.
HUETER, F. G., SHAW, J. C. & DOETSCH, R. N. (1956). J. Dairy Sci. 39, 1430.
HUGGETT, A. StG., WARREN, F. L. & WARREN, N. V. (1951). J. Physiol. 113, 258.
HUNGATE, R. E. (1943). Biol. Bull. 84, 157.
INNES, J. R. M. (1950). J. comp. Path. 60, 1.
JARRETT, I. G. (1946). Aust. J. exp. Biol. 24, 95.
JARRETT, I. G. & FILSELL, O. H. (1958). Aust. J. exp. Biol. 36, 433.
JARRETT, I. G. & POTTER, B. J. (1950). Aust. J. exp. Biol. 28, 595.
JARRETT, I. G. & POTTER, B. J. (1957). Aust. J. exp. Biol. 35, 103.
JARRETT, I. G., POTTER, B. J. & PACKHAM, A. (1956). Aust. J. exp. Biol. 34, 133.
JASPER, D. E. (1953). Amer. J. vet. Res. 14, 184.
JOHNSON, R. B. (1955). Cornell Vet. 45, 273.
KALNITZKY, G. & TAPLEY, D. F. (1958). Biochem. J. 70, 28.
KIYASU, J. Y., KATZ, J. & CHAIKOFF, I. L. (1956). Biochim. Biophys. Acta

21, 286.
KLEIBER, M. (1954). Rev. canad. Biol. 13, 333.
KREBS, H. A. (1954). Johns Hopk. Hosp. Bull. 95, 19.
KRONFELD, D. S. (1957). Cornell Vet. 47, 255.
KRONFELD, D. S. (1958). Cornell Vet. 48, 394.
LANGDON, R. G. (1957). J. biol. Chem. 226, 615.
LEWIS, D. (Personal communication.) Department of Agricultural Science, Sutton Bonington, England.
LEWIS, D., HILL, K. J. & ANNISON, E. F. (1957). Biochem. J. 66, 587.
LEWIS, D. & McDONALD, I. W. (1958). J. agric. Sci. 51, 108.
LIFSON, N., OMACHI, A. & CAVERT, H. M. (1951). Amer. J. Physiol. 166, 121.
LOTSPEICH, W. D. & PETERSEN, V. P. (1954). Amer. J. Physiol. 176, 232.
LUICK, J. R., KLEIBER, M., LUCAS, J. M. & ROGERS, T. A. (1957). Amer. J. Physiol. 191, 90.
LUKENS, F. D. W. (1938). Amer. J. Physiol. 122, 729.
McCANDLESS, E. L. & DYE, J. A. (1950). Amer. J. Physiol. 162, 434.
McCARTHY, R. E., SHAW, J. C. & LAKSHMANAN, S. (1958b). Proc. Soc. exp. Biol., N. Y. 99, 560.
McCARTHY, R. E., SHAW, J. C., McCARTHY, J. L., LAKSHMANAN, S. & HOLTER, J. B. (1958a). Proc. Soc. exp. Biol., N. Y. 99, 556.
McCLYMONT, G. L. (1951). Aust. J. agric. Res. 2, 158.
McCLYMONT, G. L. & SETCHELL, B. P. (1955). Aust. vet. J. 31, 53.
McCLYMONT, G. L. & SETCHELL, B. P. (1956). Aust. J. biol. Sci. 9, 184.
McDONALD, I. W. (1948). Biochem. J. 42, 584.
McLEAN, P. (1958a). Biochim. Biophys. Acta 30, 303.
McLEAN, P. (1958b). Biochim. Biophys. Acta 30, 316.
MALPRESS, F. H. (1958). Proc. Roy. Soc. Ser. B. 149, 362.
MARSHALL, R. A. (1949). Brit. J. Nutr. 3, 1.
MICHAUX, A. (1951). C. R. Acad. Sci., Paris 232, 121.
MILK RECORDS (1958). National milk records, Milk Marketing Board, Eastern Region.
NAKAMURA, K., SAUNDERS, P. R., WEBB, J. L., LAWSON, H. C. & THIENES, C. H. (1949). Amer. J. Physiol. 158, 269.
OXFORD, A. E. (1951). J. gen. Microbiol. 5, 83.
OXFORD, A. E. (1955). J. Sci. Fd Agric. 6, 413.
OXFORD, A. E. (1956). Coll. papers Rowett Research Institute, 12, 35.
PEARSON, O. H., HASTINGS, A. B. & BUNTING, H. (1949). Amer. J. Physiol. 158, 251.
PEETERS, G., OYAERT, W., BOUCKAERT, J. & HAECK, H. (1958). Zbl. VetMed. 5, 43.
PENNINGTON, R. J. (1952). Biochem. J. 51, 251.
PENNINGTON, R. J., & PFANDER, W. H. (1957). Biochem. J. 65, 109.
PENNINGTON, R. G. & SUTHERLAND, T. M. (1956). Biochem. J. 63, 618.
PEIRCE, A. W. (1934). Aust. J. exp. Biol. 12, 7.
PHILLIPSON, A. T. (1947). Nutr. Abstr. Rev. 17, 12.
PHILLIPSON, A. T. & CUTHBERTSON, D. P. (1956) VIIth Int. Congr. Anim. Husb., Madrid 6, p. 7.
PHILLIPSON, A. T. & McANALLY, R. A. (1942). J. exp. Biol. 19, 199.
PIGDEN, W. J. & STONE, J. E. (1952). Sci. Agric. 32, 502.

POPJÁK, G., HUNTER, G. D. & FRENCH, T. H. (1953). Biochem. J. 54, 238.
POTTER, B. J. (1952). Nature, Lond. 170, 541.
RANDLE, P. J. & YOUNG, F. G. (1956). J. Endocrin. 13, 335.
REID, R. L. (1948). Thesis, Cambridge.
REID, R. L. (1950a). Aust. J. agric. Res. 1, 182.
REID, R. L. (1950b). Aust. J. agric. Res. 1, 338.
REID, R. L. (1951a). Aust. J. agric. Res. 2, 132.
REID, R. L. (1951b). Aust. J. agric. Res. 2, 146.
REID, R. L. (1952). Aust. J. agric. Res. 3, 160.
REID, R. L. (1953). Aust. J. agric. Res. 4, 213.
REID, R. L. (1958). Aust. J. agric. Sci. 9, 788.
RICE, F. A. H. (1953). J. Amer. chem. Soc. 75, 2262.
ROGERS, T. A. & KLEIBER, M. (1956). Biochim. Biophys. Acta 22, 284.
SCARISBRICK, R. (1954). Vet. Rec. 66, 131.
SCHAMBYE, P. (1951). Nord. VetMed. 3, 555.
SCHAMBYE, P. (1955). Nord. VetMed. 7, 1001.
SCHULTZ, L. H. & SMITH, V. R. (1951). J. Dairy Sci. 34, 1191.
SEARLE, G. H., STRISOWER, E. H. & CHAIKOFF, I. L. (1956). Amer. J. Physiol. 185, 589.
SETCHELL, B. P. (1959). Biochem. J. 72, 265.
SETCHELL, B. P. & McCLYMONT, G. L. (1955). Aust. vet. J. 31, 204.
SHAW, J. C. (1955). In "The hypophyseal growth hormone: nature and actions." London & New York: McGraw-Hill.
SHAW, W. V. & TAPLEY, D. F. (1958). Biochim. Biophys. Acta 30, 426.
SOMOGYI, M. (1948). J. biol. Chem. 174, 189.
STEELE, R., WALL, J. S., de BODO, R. C. & ALTSZULER, N. (1956). Amer. J. Physiol. 187, 15.
STETTEN, D. W. & BOXER, G. E. (1944). J. biol. Chem. 155, 231.
STEWART, W. E., STEWART, D. G. & SCHULTZ, L. H. (1958). J. Anim. Sci. 17, 723.
TAYLOR, R. B. (Personal communication.) A. R. C. Institute of Animal Physiology, Babraham, Cambridge.
Van der WATH, J. G. & MYBURGH, S. J. (1941). Onderstepoort J. 17, 61.
WAKIL, S. J. & GANGULY, J. (1959). Fed. Proc. 18, 346.
WEINMAN, E. O., STRISOWER, E. H. & CHAIKOFF, I. L. (1957). Physiol. Rev. 37, 252.
WIDDAS, W. F. (1955). J. Physiol. 127, 318.
WILKINSON, J. S. (1957). Vet. Rev. Annot. 3, 69 & 4, 93.

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